DEVELOPMENT OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIERS BASED HYDROGELS FOR DIABETIC WOUND HEALING

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CERTIFICATE

بسم الله الرحمن الرحيم
In the Name of Allāh, the Most Gracious, the Most Merciful

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This is to certify that the dissertation entitled**, "Developement of Simvastatin loaded Nanostructured Lipid Carriers based Hydrogels for diabetic wound healing"**submitted by **Mr.S.Sudhakar**(M.Pharm II year),in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by him, under my guidance and supervision in the Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai – 20 during the academic year 2013 – 2014.

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DATE:

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CHAPTER – I

INTRODUCTION

CHAPTER I

INTRODUCTION

Adult skin consists of two major tissue layers: a keratinized stratified epidermis and an underlying thick layer of collagen-rich dermal connective tissue providing support and nourishment. Appendages such as hairs and glands are derived from, and linked to, the epidermis but project deep into the dermal layer. Because the skin serves as a protective barrier against the outside world, any break in it must be efficiently mended.

WOUND

A wound is defined as a defect or break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition. When circulation is reduced, blood flow lowered or dermis damaged, the wound healing takes longer time and the wound might evolve into a chronic one (Bao*et al.,* 2009).Based on the nature and repair process of wounds, they can be classified as chronic wounds and acute wounds (Boateng*et al*., 2008).

Acute wounds

Acute wounds are tissue injuries that heal within 8-12 weeks. The primary causes of acute wounds are mechanical injuries (friction contact between skin and hard surfaces), burns and chemical injuries. (Boateng*et al*., 2008).

Chronic wounds

Tissue injuries which do not succeed to heal in 12 weeks and frequently reoccur, usually result in chronic wounds. Chronic wounds arise from a disruption in the order of events of the normal wound healing process.

Broadly, chronic wounds can divide in to five groups:

- Diabetic foot ulcers
- Pressure ulcers
- Chronic venous ulcers / venous stasis ulcer
- Pyodermagangrenosum
- Vasculitis

Normal Wound healing process

To understand the causes of chronic wounds and to have a clear sight on the wound management, it is useful to discuss briefly the wound healing process.Wound healing process can be classified into five overlapping phases.

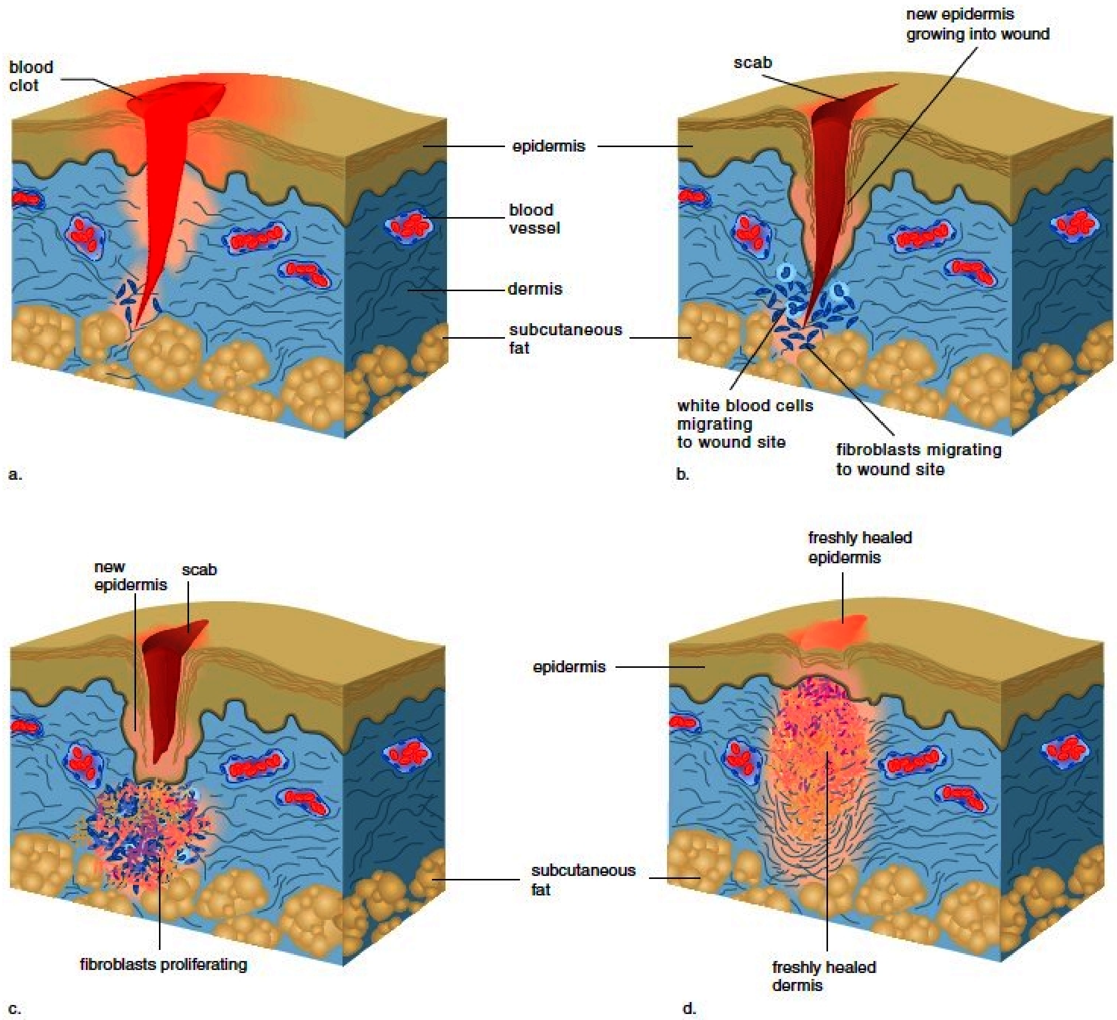
Haemostasis: When skin is injured, the first response to injury is bleeding. Bleeding is an effective way to wash out bacteria that are on the surface of skin. Exudates of cells, proteins and fibrinogen are playing an important role in activating clotting mechanism in the wound causing the bleeding to stop (Shaw and Martin, 2009). This coagulation cascade, ending with a fibrin plug and reflex vasoconstriction, achieves haemostatis. The clot dries out and creates a hard surface over the wound that protects tissues underlying (Boateng et al., 2008).

Inflammation: Inflammation is the body's reaction to injury, starts almost simultaneously with the haemostasis. It occurs from between few minutes to up to 24 minutes after injury. The wound is red, painful and moist under inflammation. The first inflammatory respond is performed by the leukocytes, specifically neutrophils, which migrate through the endothelium of the local blood vessels to the wound. The later respond is carried out by monocytes, which differentiate to macrophages in the tissues after entering by a mechanism similar to that of the neutrophils. These macrophages on their turn secrete mediators like cytokine and histamine to the inflammation site and results in vasodilatation increased capillary permeation and stimulation of pain receptors.(Muzzarelli., 2008; Rhett *et al*., 2008).and activate phagocytes to enter the wound area and engulf dead cells (Boateng*et al*., 2008).

Migration: Involves transporting growth factors in the exudates and promotes movements of epithelial cells, fibroblasts and keratinocytes to the injured area to renew damaged tissue. Cells grow over the wound, under the dried scab and gradually thicken the epithelial layer. This step lasts 2-3 days (Helms *et al.,* 2006).

Proliferation: In the proliferation phase the wound is red and can be moist but not exuding. Proliferation can last 5-20 days (Helms *et al.,* 2006). The phase of proliferation mainly includes granular tissue formation, neo-angiogenesis, and re-epithelialization.

A new dermal compound is developed named the granulation tissue which is produced by infiltration of fibroblasts, macrophages and vascular tissue fibroblasts, in response to growth factors like Plateplet Derived Growth Factor (PDGF). Neo-angiogenesis, as a result of proliferating endothelial cells, and epithelial layer covering the wound are required for the growing granular tissue. Concurrently keratinocytes migrate at the border of the wound over thegranulation tissue in a process called re-epithelialization. In this way the new outer layer of epidermis is differentiated (Muzzarelli.,2008; Boateng*et al*., 2007).



Maturation is the last step of wound healing involves the further remodulation of the granulation tissue by its constituent cells.Synthesis of structural proteins, like collagen, continues for 6 to 12 months (Muzzarelli, 2008).Collagen fibers are strengthening the skin and more capillaries are increasing the blood flow to the wound

Remolding phase constructs the ceiling. In this phase, maturation of collagen type I gets dominant while replacing collagen type III. Remolding phase can lasts up to 2 years, until 4:1 ratio of collagen I:III achieved, which is same as normal skin tissue. (Boateng*et al.,* 2008).The final scar isdetermined by formation of cellular connective tissue and strengthening of the new epitheliumand it is commonly not as strong as the skin was before injury, but 70-90% of tensile strength can be expected (Keong and Halim.,2009).

Background of complex wound healing

Both acute and chronic wounds can be classified as a complex wound if the wound has these characterizes:

- Extensive loss of the integument which comprises skin, hair and associated glands
- Infection which can result tissue loss
- Tissue death or signs of circulations
- Presence of pathology

Impaired wound healing may result from many factors, which can be classified as intrinsic and extrinsic factors.

- Intrinsic factors, or renamed local factors, contain ischemia, infection, presence of necrotic tissue and foreign bodies in the wound.
- Extrinsic factors, or external factors, include diabetes mellitus, cancer, chronic disease (chronic renal failure), steroid use, radiation damage and malnutrition. Nevertheless, chronic wounds are often a result of a combination of these factors (Izadi&Ganchi, 2005).

Diabetic Wounds

Diabetes mellitus also increases the risk of prolonged wound healing.Lots of complications are associated with diabetic foot due to the sensory, motor and autonomic neuropathy and macrovascular disease, which may lead to ulceration, infection, gangrene and eventually amputation. Commonly diabetic ulcers arise from the inability of the patient to sense pressure due to the neuropathy, what causes an increase of repeated trauma and eventually skin destruction and ulcerations. Beside the neuropathy, patients that suffer from diabetes also are at higher risk for peripheral vascular disease.

The main factors involved in delayed wound healing in diabetes,include more rapid apoptosis (cell death), reduced angiogenesis (growth of new blood vessels) and impaired lymphangiogenesis, or formation of new lymphatic vessels.

Moreover, it is assumed that diabetes mellitus is accompanied by a decrease of the immunologic defense mechanism, leading to a defect in bactericidal activity (Izadi&Ganchi., 2005). Thicker and weaker blood vessel walls and poor phagocytosis by neutrophil and macrophage are some of the reasons related for complex wounds in Diabetes. (Falanga,2003).

Wound exudate

Wound exudate can be described as generic term given to liquid produced from chronic wounds, fistulae or other more acute injuries once the haemostasis has been achieved. Exudate is actually blood free from red blood cells and components. Exudate keeps the wound moist as wound healing process gets faster in a moist environment. Exudate provides nutrition for the injured tissue and enhances the migration of epithelial cells. Exudate supplies the wound with leucocytes, which control bacterial growth and reduce the incidence of infection (Boateng et al., 2008).

In chronic wounds, an excess production of exudate can create problems. This excess can be a result of oedema caused by inflammation. Debridement is the process during which slough, eschar and exudate, bacterial biofilms and callus get removed from the wound bed to enhance the healing process. Drastic methods of debridement can be painful, therefore use of moisture-donating wound dressings will rehydrate desiccated and devitalized tissue and then, separation from healthy tissue will be easier (Fonder et al., 2008).

WOUND DRESSINGS

Dressings can be classified in a number of ways. They can be classified based ontheir

- function in the wound (antibacterial, absorbent),
- type of material employed to produce the dressing (collagen, hydrocolloid),
- physical form of the dressing (ointment, film and gel),
- traditional and modern dressings.

TRADITIONAL WOUND DRESSINGS

Topical pharmaceutical formulations: liquids such assolutions and suspensions or semi liquid materials such as ointments and creams.These formulations can be used in the initial stages of wound healing, for example asantibacterials (Boateng et al., 2008).

Traditional dressings: dry materials as cottonwool and natural or synthetic gauzes. These dressings are more used in chronicwounds and burn wounds because liquid and semi liquid dressings do not remain onthe wound over optimal time (Boateng et al., 2008).

Disadvandages of Traditional wound dressings:

- They can promote desiccation of the wound base, they bind to the wound bed and it causes pain and trauma for patients while dressing change.

- They do not provide a good barrier against bacterial growth because they are susceptible to full thickness saturation with wound fluid (Fonder et al., 2008).
- Dry wound healing process would not only delay the wound healing process, but can cause further tissue death (Fonder et al., 2008).

Some of wound dressings can cause allergic reactions when applied to the defectedarea. There are three kind of allergic reactions that can appear via wound dressings:

- Irritant reactions
- Immediate allergic reaction (contact urticaria)
- Delayed allergic reactions (contact eczema) (Goossens and Cleenewerck, 2010).

MODERN DRESSINGS

The modern dressing is basedon this concept of a moist wound environment.They can stimulate collagen synthesis and promote the angiogenesis for the achievement of effective oxygen circulation, regeneration of cells and tissues, and for the lower bacterial load.

Hydrocolloid dressings: Hydrocolloid dressings of colloidal materials associated with other materials, for example elastomersand adhesives. These colloidal materials are gel forming agents, such as CMC, gelatin andpectin. They appear as thin films and sheets and are applied on mildly exuding wounds, suchas pressure sores, minor burns and traumatic injuries. (Boateng*et al.*, 2007).

Alginate dressings: Alginate dressingsappear as freeze-dried porous sheets (foams) or as flexile fibers.Alginates containcalcium and sodium salts of alginic acid. An ion exchange occurs between calciumfrom alginate and sodium from wound fluid, forming sodium-calcium alginate whichgives a gelatinous mass and can keep the moist environment (Fonder et al., 2008). Generally accepted,alginate dressings are used for moderately to heavily exuding wounds (Boateng*et al.*, 2007).

Semi-permeable adhesive film dressings: These wound dressings are made from nylon derivatives that can be applied for moistwound healing. They can be applied mostly for shallow wounds (Boateng*et al*., 2008)

Films: Films are semi-occlusive, permitexchange of oxygen and water between wound bed and outside and at the same timethey are impermeable to bacterial and liquid contaminants. They provide amoist environment by vapour transmission only. If they are applied to wounds withheavy exudate, they can cause fluid trapping (Fonder *et al.*, 2009).

Foam dressings: These dressings are porous polyurethane foams or polyurethane foam films make provision for thermal isolation and are easily applicable. They arehighly absorbent and preferred to gauze in term of pain reduction, patientacceptability and nursing time (Boateng*et al*., 2008).

Compression dressings: used in the treatment of chronic venous insufficiency. These dressings exert theireffect by reducing fluid extravasation into the extracellular space to achieve better diffusion ofoxygen and nutrients to the wound (Izadi&Ganchi, 2005).

HYDROGEL DRESSINGS

Hydrogels are swellable hydrophilic materials.Hydrogels can function as a film on wounds and in addition keep the wound moist. Hydrogels can be washed off by water when change is needed. This will keep the newly made granulation tissue more intact than when removing a dry bandage (Chaby*et al.,* 2007). Hydrogels can be produced in two shapes, amorphous or solid sheet/films. If hydrogels are applied to the wound as gels, they need a second cover such as gauze. On the other hand if they are applied as films to the wound, they can be used both as a primary and secondary dressing(Boateng et al. 2008).The main features of hydrogels influencing their use in wound treatment are:

- Shape stability and softness similar to that of the soft surrounding tissues
- Chemical and biochemical stability
- High permeability for water-soluble nutrients and metabolites across the biomaterial Tissue-interface (Kopecek, 2009).

Some additional advantages of hydrogels as wound dressings are:

- Help to the rehydration of dead tissues and increase the healing
- Suitable rheological properties
- Convenience in handling&Ease of application
- Good biocompatibility due to their high water content (Kopecek, 2009).
- Hydrogels can be the most suitable dressing in debridement stage of a chronic wound (Vaneau et al., 2007).
- Are suitable for cleansing of dry, sloughy or necrotic wounds
- Do not react with biological reacts ,nonirritant and non-adherent
- Cool the surface of the wound

Hydrogels should be used for dry or low level of exudate wounds. The excess moisture can lead to maceration of skin. Hydrogels can be applied and removed with minimal trauma and pain from wound bed. Because of the cooling effect that hydrogels have on wound bed, they can give a relief feeling to patients (Fonder et al., 2008). These dressings can be used for the treatment of chronic leg ulcers due to their pain reducing characteristics*(Boateng et al., 2007).*

In general, hydrogels can be prepared from either synthetic polymers or natural polymers. An ideal material to be applied to wound should beNontoxic, Biocompatible, Enhance cellular interaction and tissue development,biodegradble and bioresorbable (Huang and Fu, 2010).

Hydrogels of natural origin

Chitosan has been utilized for many medical and pharmaceutical preparations. Properties such as being biocompatible, non-toxic and soluble in weak acids make it an excellent wound dressing. It is positively charged, is strong tissue adhesive and forms gel easily. It has proven to promote adhesion and proliferation in wounds. (Yang *et al.,*2008). And also chitosan can enhance function of leukocytes, macrophages and fibroblasts to enhance granulation and rebuilding tissue (Huang and Fu, 2010).

Hydrogels of synthetic origin

The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand mechanical strength provides the durability as well. These two opposite properties should be balanced through optimal design (Tabata, 2009). Biocompatibility of synthetic hydrogels is on the other hand not as good as for some hydrogels of natural origin, for example chitosan gels (Keong and Halim, 2009).

Chitosan HG on wound healing

- Chitosan, By reducing the trans-epidermal water loss, it increases water-binding capacity and skin moisture and produces durable moisturizing effect on the skin.
- It also improves the sensorial parameters and the dermatological compatibility of formulations (Dodane and Vilivalam, 1998; Muzzarelli and Muzzarelli, 1998; Klingels*et al*., 1999).
- The film-forming ability of chitosan assists in imparting a pleasant feeling of smoothness to the skin and in protecting it from adverse environmental conditions and consequences of the use of detergents.
- Chitosan was found to be superior to hyaluronic acid as far as lasting hydrating effects are concerned (Muzzarelli and Muzzarelli, 1998).
- Chitosan has been used as an antibacterial and antifungal agent.

Chitosan improves wound healing

Catalysis of chitosan degradation is known to be caused by lysozyme, an enzyme transported to the wound sites by the inflammatory cells. The abundant lysozyme at the wound site would break chitosan to the active N-acetyl-D-glucosamine dimer and provide for its sustained release. Because lysozyme acts slowly on chitosan, chitosan becomes a continuous source of N-acetyl- D-glucosamine dimers as long as the wound contains inflammatory cells releasing lysozyme. The inflammatory reactions and therefore the release of lysozyme, continues until the wound is finally healed.

Classification of hydrogels

Chemically cross-linked hydrogels:

Radical polymerization is usually applied to make these polymers. When these types of hydrogels come in contact with H_2O molecules, they begin to swell up and spread their network (Jagur-Grodzinski, 2009).

Physically cross-linked hydrogels:

Physically cross-linked hydrogels do not need introduction of an external cross-linking agent. Cross-linking agents are usually nondegradable and can be toxic and a removal of their residuals may be needed before they can be used in biomedical or pharmaceutical purpose. The physically crosslinked hydrogels are usually biodegradable. Their amorphous hydrophilic phase is held together by highly ordered aggregated chain segments held together by secondary molecular forces such as hydrogen bonding, Van der Waals forces orhydrophobic interaction (Jagur-Grodzinski, 2009).

There are several other classifications for hydrogels. They can also be classified based on

- nature of the network: homopolymer, copolymer, interpenetrating, or double networks;
- physical structure: homogeneous (optically transparent), microporous, and macroporous hydrogels;
- fate in the organism: degradable and nondegradable hydrogels (Kopecek, 2009).
- stimuli-sensitivity: Temperature responsive hydrogels, pH responsive hydrogels, Analyte responsive hydrogels

SIMVASTATIN ON DIABETIC WOUND HEALING

Simvastatin, a HMG-CoA reductase inhibitor, have beenshown to exhibit important immunomodulatory effects independent of lipid lowering (Kwak B*et al*., 2000). simvastatin promote healing. By the following machanisms,

- The basis of wound healing is the fact that statins increase the activity of eNOS& angiogenesis. There is a positive correlation between eNOS& angiogenesis;increase in which favors wound healing.Angiogenesis is required for restoration of blood flow for growing tissue.This is the basis of wound repair since it is essential for the supply of oxygen & other nutrients required in the cellular & biochemical process of the repair.
- The other mechanisms involved in the pro-healing activity of statins include proper stimulation of the endothelial cell migration, proliferation & differentiation; since i*n vitro* endothelial cell sprouting assays confirmed that eNOS is required for the same.
- Inhibition of NAD(P)H oxidase leading to suppression of superoxide formation & oxygen free radical scavenging by statins could also help in promoting the healing by reducing oxidative damage.

The objective of the present study was to develop Hydrogels loaded with simvastatin for the treatment of chronic wounds associated with diabetes melitus.

CHAPTER – II

A REVIEW ON
NANOSTRUCTURED LIPID CARRIERS

CHAPTER II

A REVIEW ON NANOSTRUCRED LIPID CARRIERS

A Novel Generation of Solid Lipid Carriers

Many topical and dermatological formulations may susceptible to various changes if stored incorrectly. Emulsions may cream and crack,suspensions can agglomerate and cake, and ointments and gels may bleed as their matricescontract to squeeze out mobile constituents. High temperatures can produce or accelerate suchadjustments. Heat may alter the phasedistribution of cream and may even crack the emulsion. For milky white microemulsion creams or lotions it is difficult to determine ifthe final product requires additional mixing time to properly disperse or solubilize the drug.

To overcome such problems of these multiphase systems, a new approach would be thepreparation of lipid nanoparticles for drug entrapment and furtherincorporation into a monophasic system such as hydrogels.

Lipid nanoparticles were developed in the last decade of the last century as alternative carrier system to emulsions, liposomes and polymeric nanoparticles (Lucks and Müller, 1996; Mehnert and Mäder, 2001; Müller et al., 2000a,b, 1995). Drawbacks associated to the traditional colloidal systemsare the presence of solvent residues left over from production, the cytotoxicity of the polymers, and the lack of low-cost, qualified large-scale production units yielding a product of a quality acceptable by the regulatory authorities.

In the majority of the pharmaceutical formulations intended for topical and dermatological therapy the drug molecules are totally dissolved in a liquid phase of oil-in-water (o/w) or water-in-oil (w/o) emulsions. A major disadvantage of emulsions and liposomes is the lack of protection for chemically labile drugs, in addition drug release takes place as a burst (emulsions) or at least relatively fast (from liposomes) due to the low viscosity of the inner phase of the afore-mentioned systems.

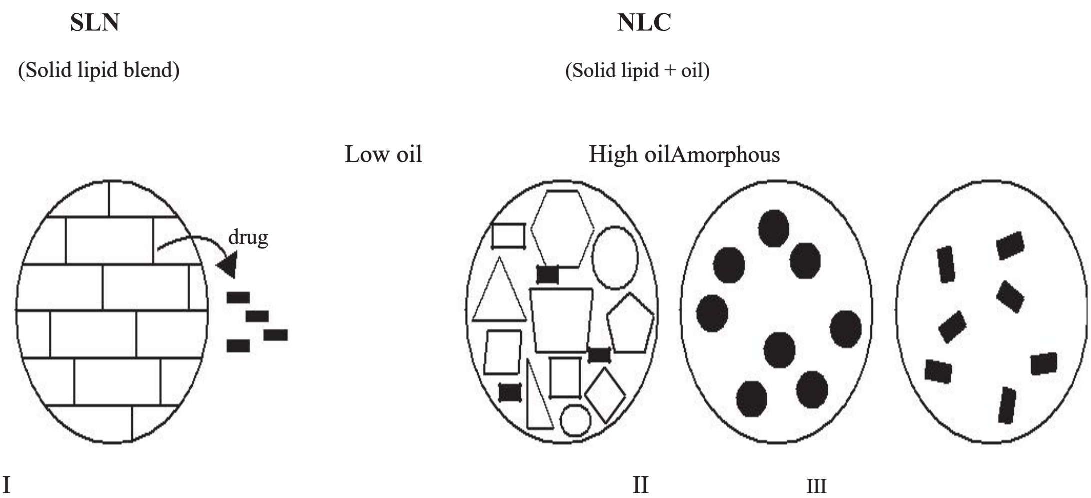
As an alternative, solid lipid nanoparticles (SLNs) were developed by exchanging the liquid lipid (oil) of the emulsions by a solid lipid (Muller R.H *et al.*,2000) matrix identical to polymeric nanoparticles(Siekmann B*et al.*, 1998) whichmay exhibit sustained releaseproperties. Another clear advantage of SLN compared to polymeric nanoparticles is the availability of large scale production units. However, there are also three limitations of the SLN system: limitation of drug load by the solubility of the drug in the solid lipid,drug expulsion phenomenon when lipid crystallizes to the stable β-form, particle concentration in the aqueous dispersions ranging from about 1% to a maximum of only 30%. The aim of the research was to overcome these limitations by creating a new lipid carrier, the NLC.

Nanostructured lipid carriers (NLCs) are a second generation of SLNs, which have a solid matrix mixed with a liquid lipid (oil) to form an unstructured matrix that helps increase the drug loading capacity of nanoparticles, avoids or reduces drug expulsion from the matrix during storage and giving more flexibility for modulation of drug release.This approach is achieved by mixing solid lipids with liquid lipidsinstead of highly purified lipidsprferebly in a ratio of 70:30 upto a ratio of 99.9:0.1. The result is a less ordered lipid matrix with many imperfections, which can accommodate a higheramount of drug (Müller, R.H *et al.*, 2004), (Müller, R.H *et al.*, 2002).The overall solid content of NLC can be upto 95% (w/w) (Muller et all., 1999).(Müller, R.H *et al.*, 2002), (Müller, R.H *et al.*, 2003).

TYPES OF NLC

Imperfect type NLCs: In type I, solid lipids and liquid lipids (oils) are blended. The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs .

Multiple type NLCs: When mixed with large amount of oil, the lipid was found to solubilize certain drugs which were not soluble otherwise. The multiple oil/fat/water, drug can be accommodated in the solid, but at increased solubility in the oily parts of the lipid matrix. At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase, leading to phase separation that means precipitation of tiny oily nanocompartments.



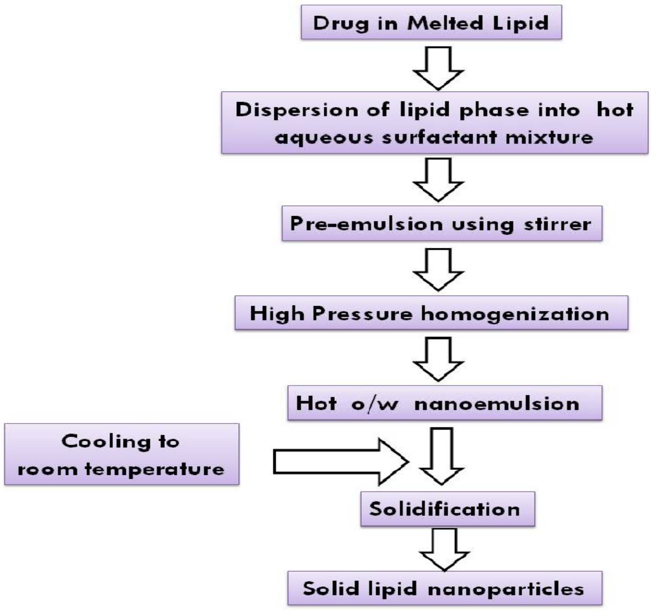
Amorphous NLC: It was created to reduce drug expulsion by mixing special lipids like hydroxyl octacosanylhydroxystearate and isopropyl myristate.In type III, lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in an amorphous state . The absence of crystallization avoids drug expulsion by crystallization.

Methods of preparation for NLC

High Pressure Homogenization

High pressure homogenizers push a liquid with highpressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluidaccelerates on a very short distance to very high velocity (over 1000 km/h).

Hot homogenization



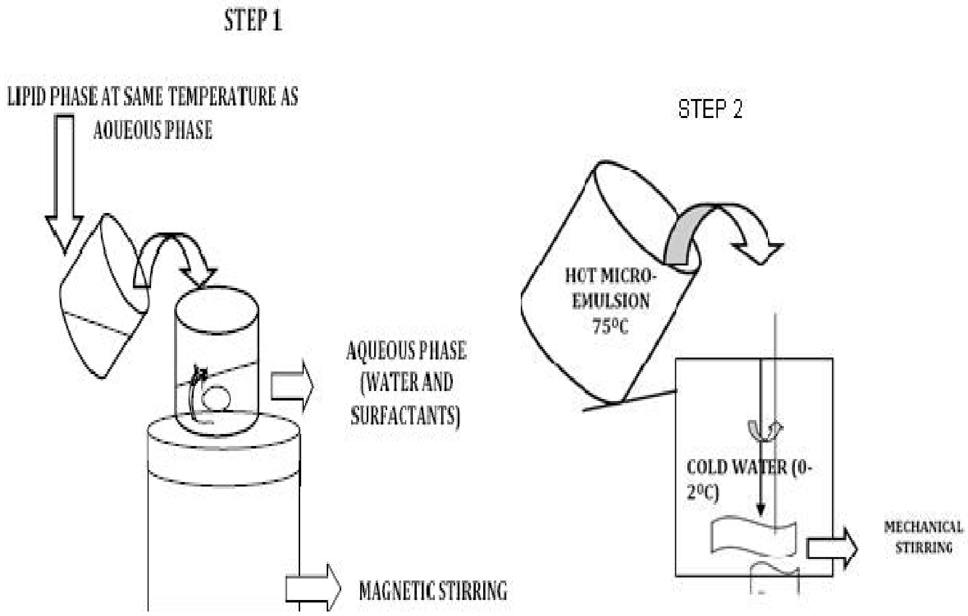
Cold homogenization

Cold homogenization overcome various problems associated withhot homogenization such as temperatureinduced drug degradation, drug distribution into the aqueous phase during homogenization.In cold homogenization the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a presuspension. Then this pre-suspension is homogenized at or below roomtemperature, the gravitation force is strong enough to break the lipid microparticlesdirectly to solid lipid nanoparticles.

Solvent emulsification-diffusion method

The particles with average diameters of 30- 100 nm can be obtained by this technique. Voidance of heat during the preparation is the most important advantage of thistechnique

Microemulsion based method



Supercritical fluid

NLC can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) was the good choice as a solvent for thismethod.7

Spray drying method

It's an alternative procedure to lyophilization in order to transform an aqueous NLC dispersion into a drug product. But his method can cause particle aggregation due to high temperature, shear forces and partial melting of the particle.

Double emulsion method

In double emulsion technique the drug (mainly hydrophilic drugs) was dissolved inaqueous solution, and then was emulsified in melted lipid. This primary was stabilized by stabilizer. Then this stabilised primary emulsion was dispersed in aqueous phase containing hydrophilic emulsifier. Thereafter, the double emulsion was stirred and was isolated by filtration.

Precipitation method

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

Film-ultrasound dispersion

The lipid and the drug were put into suitable organic solutions, after decompression, rotation and evaporation of the organic solutions, a lipid film is formed, then the aqueous solution which includesthe emulsions was added. Using the ultrasound with the probe to diffuser at last, the NLC with the little and uniform particle size is formed.

Routes of Administration

The research activities in SLN and NLC in the last two decades focussed mainly on pharmaceutical nondermaladministration routes, i.e. parenteral (Blasi et al., 2007; Joshi and Müller, 2009; Wissing et al., 2004), peroral (Muchow et al., 2008; Müller et al., 2006; Sarmento et al., 2007), ocular (Attamaet al., 2007; Ugazio et al., 2002) and pulmonary (Liu et al., 2008) administration.

Advandages

A clear advantage of the use of lipid particles as drug carrier systems is the fact that the matrix is composed of physiological components, i.e. excipients with generally recognized as safe (GRAS) status for oral and topical administration, which decreases the danger of acute and chronic toxicity. It can also be pointed out that NLC are low cost products (Müller, R.H., 2005). In fact, the excipients and production lines are relatively cheap and the production costs are not muchhigher than those established for the production of parenteral emulsions (Wissing, S.A., 2004, Müller, R.H., 2004).

Advantages of NLC as a Topical Drug Delivery system

During the last 5 years SLN and NLC have been intensively investigated for dermal application because of many positive features that have been reported after their application to the skin (Müller et al., 2007, 2002b).

Due to the lipid matrix, the small particle size and related adhesive properties, the residence time of NLC on the skin is prolonged. There is a lipid interaction of the particle matrix with skin surface lipids, affecting drug absorption (Lombardi Borgia et al., 2007; Santos Maia et al., 2002).

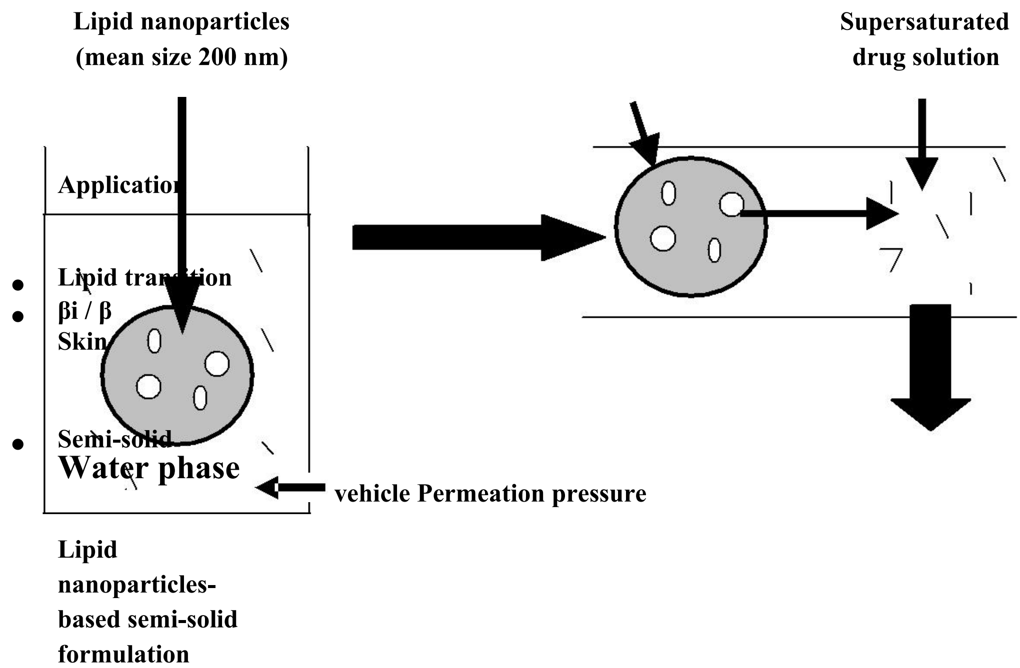
Occlusive properties, increase in skin hydration, modified release of actives, targeting effects to specific skin strata especially when the drug is located at the particle surface are also positive features of lipid nanoparticles (Müller et al., 2007).

NLC were found to enhance the skin penetration of several dermally applied drugs(Lombardi Borgia et al., 2005; Souto et al., 2004; Stecova et al.,2007).

Concerning aqueous SLN andNLC dispersions, once composed of physiological lipids they might be less comedogenic thaneither creams or lotions. It will therefore be possible to develop systems that are non-toxic,non-irritating, non-comedogenic and non-sensitizing and formulating pharmaceutically and/orcosmetically elegant topical formulations based on NLC.

Drug release profile from NLC based HG

Drug penetration into certain layers of the skin can be achieved using NLC as a consequence of the creation of a supersaturated system (Müller, R.H *et al.,* 2002).



Phenomenon of triggered drug release and supersaturation effect of lipid nanoparticles entrapped into a dermatological formulation, i.e. semi-solid vehicle (modified after Müller *et al.* (Müller, R.H., 2002).

Incorporation of lipid nanoparticles such as NLC into topical formulations (gels) can be performed to create supersaturated systems to enhance the permeation of drugs across membranes such as skin without affecting the ultrastructure of the stratum corneum.. The increase in saturation solubility will lead to an increased diffusion pressure of drug into the skin.

The developed semi-solid systems consist of a three-dimensional vehicle (hydrogel) which encloses and it is interpenetrated by an aqueous NLC dispersion. During storage, the drug remains in the lipid matrix because these particles preserve their modification. After application onto the skin, the increase in temperature and water loss observed lead to transformation to a more ordered lipid modification and are responsible for drug expulsion from the lipid matrix. The drug is expelled into the semi-solid vehicle (hydrogel) already saturated with drug and thus leading to supersaturation. This phenomenon increases the thermoactivity and leads to drug penetration into the skin.

Concerning the release properties of the developed formulations, the jellifying agent can modify the observed diffusivity of the drug from lipid nanoparticles by either mechanically impeding its movement or by adsorbing the drug on the polymer surface. Since the actual pathway for diffusion in gels is through the fluid phase, the factors which affect diffusivity in pure liquid phases similarly control diffusion within the gels.

Polymeric systems such as hydrophilic gels, express non-Newtonian pseudoplasticbehaviour,. As the degree of pseudoplasticity increases, easiness of spreadability augments (das Neves et al., 2009).

The scope of this studywas toformulation ofsimvastatin loaded nanostructured lipid carrier and further incorporated into chitosan hydrogel and to determine the effect of NLC based hydrogel of simvastatin on the extent of wound healing acceleration. To ascertain simvastatin's wound healing acceleration superiority, simvastatin loaded NLC based HG will becompared with plain simvastatin HG and NLC based HG's without drug.

CHAPTER – III

LITERATURE REVIEW

CHAPTER III

LITERATURE REVIEW

- **Prachi B.Shekhawat*et al.*, 2013,** preparedClotrimazole loaded Nanostructured lipid carrier for topical delivery by hot homogenization method using Stearic acid as solid lipid, oleic acid as liquid lipid. Combination of four types of surfactants : Tween 80, Polaxamer 188, Sodium Lauryl Sulphate (SLS) and lecithin were used to stabilize NLC dispersion. The penetration of Clotrimazole from CM-NLC formulation into cadaver skin was evaluated in vitro using franz diffusion cell. The CM-NLC formulations would significantly increase the accumulative uptake of CM in skin over the marketed gel and showed a significantly enhanced skin targeting effect.
- **GajananShinde,*et al.*, 2013,** preparedMethoxalen loaded NLC by hot homogenization followed by High speed homogenization technique, using Glyceryldistearate, Compritol ATO 888 and Stearic acid as solid lipid, Ethyl oleate as liquid lipid and Pluronic F-68 as surfactant .The mean particle size, polydispersity index (PDI), zeta potential and entrapment efficiency were found to be 258.2 nm, 0.183, -20.9 mV, 97.45 % respectively. In vitro release studies demonstrated that NLC formulations possessed a controlled release over a period of 24 hrs. The pH, Viscosity, Spredability, Refractive Index, Drug content, Skin permeation, Drug deposition of prepared NLC based gel were characterised.NLC based gel was not shown skin irritation and shown prolong release up to 24 hr. There was insignificant change in pH, Drug content and In vitro release indicating the developed NLC were fairly stable.

- **Gajanan S Sanap*et al*., 2013,** designed and evaluated Miconazole Nitrate Loaded Nanostructured Lipid Carrier for improving the antifungal therapy.NLC designed for topical application by hot high pressure homogenization technique. The lipid nanoparticles are incorporated in gels for convenient topical application. The ln vitro and Ex vitro skin permission data indicate that MN loaded NLC bearing hydrogel provides sustained release of MN.
- **Anne des Rieux*et al*., 2013,** studied mechanism of transport of Saquinavir loaded Nanostructured Lipid Carriers across the intestinal barrier. The size and amount of surfactant in the NLC influenced SQV's permeability, transcytosis pathway efflux of SQV by P-gp. An NLC of size 247 nm and 1.5% W/V surfactant circumvented P-gp efflux.
- **Sachin S.Salunkhe*et al* ., 2013,** developed Nanostructured lipid carrier for topical delivery of Idebenone by using solvent precipitation method.Compriol 888 ATO, Captex 500 P and ethanol were selected as solid lipid, liquid lipid and solvent respectively.Labrasoland TranscutolP as surfactant and co-surfactant had given stable formulations on the basis of HLB required for stabilization. Animal model studies such as in vitro skin permeation, skin deposition, sun protection factor determination studies gave proof for effective delivery of drug by incorporating into NLC.
- **ShaimaaElkadi*et al*., 2013,** designed and Optimized the self nano-emulsifying drug delivery systems of Simvastatin. Oils, surfactant and co-surfactant composition was optimised using drug solubility, tertiary phase diagram, system stability and droplet size distribution

studies.Results revealed that 10% relatively polar oils, 60% Cremophore RH 40, (surfactant) and 30% Transcutol HP (co-surfactant) acquired good nano-emulsification properties either in liquid or tableted forms.

- **Himeshsoni*et al.*, 2013,** formulated and optimized hydrogel systems containing rutin0.025% w/w which can improve the therapeutic efficacy of poor water soluble drugs. Hydrogels were prepared using Carbopol934 and HPMC in different concentrations of 2:1, 1:1, 1:2 along with methanolic dispersions of rutin. Hydrogel can sustain the release of rutin revealed that drug remains localized for a longer period of time, thus enabling drug targeting to the skin and feasibility of using a dermatological formulation to improve skin wound healing, inflammation and prevent microbial infection.
- **Almeida, Mariana Mandelli*et al.,* 2013,** prepared Nanostructured Lipid Carriers containing Ursolic acid in cosmetic formulation by High Pressure Homogenization and evaluated physical and chemical stability of prepared NLC formulation by Accelerated Stability test under varying conditions of temperature and luminosity. Based on the results, the ursolic acid in the NLC form achieved a better physical and chemical stability compared to ursolic acid in the free form in both variation of the viscosity and pH.
- **GuissiNida El Islem*et al.,* 2013,** prepared and characterised low crystalline and more swollen Nimodipine loaded Nanostructured Lipid Carriers and Solid Lipid Nanoparticles in the presence of Electrolytes by modified solvent injection method using Glyceryl mono stearate as solid lipid, Soyabean oil as liquid lipid and Polaxamer 188 and tripolyphosphate as stabilizers. As a result, all developed formulations showed a narrow Polydispersity Index lower than 0.3, good physical stability and Zeta potential value ranging from -34mV to -

66mV approximately. SLN and NLCs prepared in the presence of Electrolytes have shown higher Entrapment efficiency (upto 87.52%) , higher drug loading(4.19%) and increased drug release rate up to 96.7%.

- **Jun Asai*et al.*, 2012,** done aresearch on "Topical Simvastatin shown to accelerate wound healing in diabetes". Factors involved in delayed wound healing in diabetes include more rapid apoptosis, reduced angiogenesis and lymphangiogenesis.This study shows that topical Simvastatin significantly accelerates wound recovery by increasing both angiogenesis and lymphangiogenesis. The favourable effects of Simvastatin on lymphangiogenesis are due both to a direct influence on lymphatics and indirect effects via macrophages homing to the wound.
- **Michel FleithOtuki*et al.*, 2012,** prepared Simvastatin ointment for the treatment of skin inflammatory conditions. The aim of the study was to investigate the activity of Simvastatin in acute and chronic inflammatory models of mice. The result confirmed the anti-inflammatory activity of Simvastatin when applied topically in both acute and chronic models of skin inflammation. Besides, the incorporation of 1% Simvastatin in an ointment vehicle maintained its anti-inflammatory activity and prevented problems associated to the skin barrier disturption observed with a preparation of a higher concentration (3%).
- **Santhoshkumar R. Jeevangi*et al.*, 2012,** compared anti-inflammatory activity of Lovastatin, Simvastatin, Atorvastatin and Rosuvastatin on acute and chronic inflammation in animal models. All the statins showed significant anti-inflammatory activity in the present study in both acute as well as

chronic models of inflammation. The anti-inflammatory activity of the 4 statins was significant on comparision with Diclofenac. Lovastatin and Simvastatin demonstrated 10-20% more anti-inflammatory activity than Atorvastatin and Rosuvastatin.

- **Dilip Patel *et al.,* 2012,** done a research on Nanostructured Lipid Carriers based Gel for the topical delivery of Aceclofenac. Strearic Acid as the solid lipid, Oleic Acid as the liquid lipid, Pluronic F68 as the surfactantant and phospholipon 90G as the co-surfactant were used. NLCs were prepared by melt-emulsification, low-temperature solidification and high speed homogenization methods. The NLC dispersion was suitably gelled and assessed for invitro permeation. Finally NLC based gels were found to possess superior (almost double) the anti-inflammatory activity with rapid onset of action and prolonged duration of action compared to the marketed gel.
- **A.C. Silva *et al.*, 2012,** developed two different solid lipid Nanoparticles (SLN) Loaded hydrogel as potential carrier for oral transmucosal delivery of Risperidone. The rheological behaviour of semisolid formulations revealed a plastic flow with thixotrophy. The SLN remained within thecolloidal range after the preparation. In vitro release studies revealed a more pronounced RISP release after SLN hydrogel entrapment when compared to the dispersion alone. In addition pH dependent release observed as well. The predicted in vivo RISP release mechanism was Fickian diffusion alone (or) combined with erosion.
- **Evren it Gokce*et al.*, 2012,** compared Resveretrol loaded SLN versus NLC. Nanoparticles were prepared by high shear homogenization using Compritol ATO (solid lipid), Miglyol (liquid lipid), Polaxamer 188, and

Tween 80. NLC formulation containing 255 mg of Compritol and 45 mg of Miglyol (15% of the lipid phase) was the most promising formulation. The drug EE was 18% higher in NLC system. Ex vivo skin studies revealed that NLC are more sufficient in carrying the RSV to the epidermis. When the two system compared, NLC penetrated deeper into the skin. RSV loaded NLC with smaller PS and higher drug loading appears to be superior to SLN for dermal applications.

- **SiripornOkonogi*et al.*, 2012,** carried out a project on effect of surfactant on Lycopene loaded Nanostructured lipid carrier. The preparation of the NLC was achieved by means of high pressure homogenization. The result of this study demonstrate that the contact angle as a key tool to overcome the small size of NLC . It was found that small contact angle gave NLC with small size. Plantacare 1200 gave Lycopene loaded NLC with smaller size, high zeta potential and narrower size distribution.
- **Sahu M.K *et al.*, 2012,** formulated & characterized the topical Nanostructured Lipid carrier gel of Fluriprofen and its comparison with micellar gel preparation. The Fluriprofenloaded NLC prepared by solvent diffusion method. The encapsulation efficiency and drug loading of Fluriprofen loaded NLC was in the range of 78.28 - 72.13 % and 21.84 - 20.68 % respectively. The particle size and zeta potential of prepared formulation was found to be in the range of 120.48 - 280.12 nm and -22.32 to -21.18 respectively. FLUNLC showed better blood concentration in vivo (36.95 ± 1.23 µg.min/ml) than marketed fluriprofen formulation (13.55 + 0.63) representing greater plasma level of fluriprofen after administration.

- **H.M. Nguyen *et al.,* 2012,** performed a project on enhanced payload and photo-protection for pesticides using Nanostructured lipid carriers with corn oil as liquid lipid. Deltametherin loaded Nanostructured lipid carrier and solid lipid nanoparticles were prepared by hot homogenization and sonication with different ratios of corn oil (Liquid lipid) and Bess wax (solid lipid). Compared to solid lipid nanoparticles (SLN), NLC gave a higher payload (approximately 1.79 times) with high encapsulation efficiency (83.6%) and higher photo protection in both direct photolysis (1.8 times) and in indirect photolysis (1.37 times).
- **Premanand A.Nighojkar*et* *al.*, 2012,** formulated BetaMethasoneDipropionateloaded Nanostructured lipid carrier enriched gel by hot homogenization followed by ultrasonication method. BMP-NLC enriched gel using Tyloxopol as surfactant andPhosphatidylcholine as an emulsifier with optimum concentration of 3.0% and 2.0% shows least particle size (342.6nm), high entrapment efficiency (85.50%) and average drug content (0. 803 mg/ml). Cumulative percentage of drug release of BMP-NLC enriched gels showed higher drug permeation (80. 5%) through excised rat skin at the end of 24 hrs and stable at room temperature.
- **Huizhou*et al*., 2012,** encapsulated Oxaliplatin in Nanostructured lipid carriers by hot high-pressure homogenization method. Differential scanning colorimetry and x-Ray diffraction studies revealed that the drug was dissolved in the blend of Glyceryl mono stearate and medium chain trigiylerides with amorphous forms. The in vitro release experiments of

NLC exhibited biphasic drug release pattern with burst release at the initial 2 hr and prolonged release afterwards.

- **Samantha C. Pinho*et al.*, 2012,** developed a lipid particle for β-carotene using blend of 70 % trisearin and 30 % sunflower oil (6 % total lipid)by hot pressure homogenisation and stabilized with hydrogenated soylecithin and tween 80 (3% total surfactant).Two types of particles were produced, using 1 and 2 passage in the homogenization step. The systems in terms of size distribution although the particles produced with 1 passage were slightly more efficient at protecting the beta-carotene from degradative process and also suffered less microstructural alteration after 4 months.
- **UsamaFarghalyAly*et al.*, 2012,** prepared and evaluated Atorvastatin in different type of gels including hydroalcoholic, hydrogel, microemulsion, anhydrous and alcoholic gel bases. The prepared gels were evaluated for physical appearance, rheological behaviour, drug release through a standard cellophane membrane and wound healing power in Streptozotocin induced diabetic male albino rats. The rank order of gel formulations based on their maximum release was alcohol > hydrogel >hydroalcoholic> anhydrous >microemulsion gel base. Results of in vivo wound studies revealed that the anhydrous base showed the highest parent wound contraction with complete wound closure and epithelisation was observed on 7^{th} day of wound contraction.
- **JaberEmami*et al.,* 2012,** formulated Cholesterol Nanostructured Lipid Carriers (NLCs) with various Oleic Acid content loaded with Paclitaxel by solvent emulsification diffusion method using a Taguchi design. Size, Zeta Potential, Entrapment Efficiency, drug loading and release percent of NLC

were measured. The results indicated that most effective factor on size were Oleic Acid and Surfactant content. Zeta Potential was most affected by drug content. Drug to lipid weight ratio was the most effective factor on entrapment efficiency and drug release from NLC.

- **Dubey A.*et al.*, 2012,**reviewed nanostructured lipid carrier : A novel topical drug delivery systems. This review concentrates on development of nanostructured lipid carrier for topical applications. The lipid nanoparticles SLN and NLC are carrier systems with good perspectives to be marketed very successfully. The reason is that they were developed considering industrial needs e.g. Scale up, qualification and validation, simple technology, low cost, tolerability etc. The smart NLC as the new generation offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms such as creams, tablets, capsules, and injectabes.
- **Sahu*et al.*, 2012**, reviewed Nanostructured Lipid Carrier : The second generation of solid lipid nanoparticles. SLN was referred to alternative carrier system to traditional colloidal systems such as liposomes, emulsions, and polymeric nanoparticles due to its exceptional stability, scaling up potential and bio-compatible components. A new generation of nanostructured lipid carrier consisting of a lipid matrix with a special nanostructure has developed with improved drug loading and storage.
- **Meghana S. Kamble*et al.*, 2012,** reviewed Solid lipid nanoparticles and nanostructured lipid carrier. To overcome the stability and drug expulsion problems of SLN, The NLC had emerged. The highly unordered lipid matrix structure of NLC improved drug encapsulation and stability also presenting controlled and targeted drug release. NLC can be administered via oral, ocular,

pulmonary, and intravenous routes. The present reviews the types of NLC, preparation, methods, and characterization of SLN and NLC.

- **MahiranBasri*et al.*, 2011,** done a project on " an improved method for the preparations of Nanostuctured lipid carriers containing heat sensitive boiactives". A previously established high-temperature high-pressure homogenization technique involved in the preparation of the NLCs can cause degradation of heat sensitive active compounds during the preparation of NLC. In this work, modified methods A and B were developed on the basis of initial location of surfactants and fast cooling step after homogenization which enhance the chemical stability of heat sensitive compounds and physical stability of NLCs significantly compared to the previously established method.
- **CheeWun How *et al.*, 2011,** reviewed Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilized with polysorbate 20 and polysorbate 80. NLC80 were produced by high-pressure homogenization technique, stabilized with polysorbate 20 and polysorbate 80, respectively. Photon correlation spectroscopy showed that the average size of NLC80 and NLC20 were 102.8 ± 0.1 and 261.63 ± 8.56 nm, respectively, and their zeta potentials were -23.93 ± 0.75 and -30.57 ± 0.06 mV, respectively. The results suggest that NLC80 is a more stable formulation. The melting point depression of NLC80 was 5.71°C below bulk lipid's melting point (61.56°C), while NLC20 exhibited two melting points at 54.80 and 59.10°C. These findings suggest that polysorbate 80 was a better dispersing agent for NLC than polysorbate 20. The physicochemistry properties of the NLCs aregreatly influenced by the type of surfactant used.

- **PardeikeJ. *et al*., 2011,** developed an itraconazole loaded nanostructured lipid carrier formulation for pulmonary application. Selecting lipid matrix and stabilizers based on solubility and miscibility investigations and contact angle measurement respectively can help formulating NLC with good stability and high entrapment efficiency. Based on this Precirol and Oleic acid in the ratio of 9:1 as lipid matrix, 2.5% EumulginSLM 20 as stabilizer is used. An entrapment efficiency of 98.78% was achieved with Narrow size distribution and sufficient stability.
- **Gambhire M.S *et al.,* 2011,** formulated Simvastatin loaded solid lipid Nanoparticles by pre-emulsion ultra sonication technique and optimized using Box. Behnken design. The results of optimized formulation showed average particle size of 245 nm and drug entrapment of 75.52% and low in vitro release of 37.08% than dispersion of pure drug (97.2%).
- **MakarandGrambhire*et al.,* 2011,** studied bioavailability assessment of Simvastatin loaded solid lipid nanoparticles after oral administration. Simvastatin SLN developed using Compritol 888 ATO by pre-emulsion followed ultrasonication. Stable Simvastatin SLN having mean size of 245 nm and % entrapment of 72.52% were formulated. The relative bioavailability of Simvastatin and Simvastatinhydroxy acid from SLN were increased by ~164% and ~207% respectively compared with reference Simvastatin suspension.
- **LacerdaS.P. *et al*., 2011,** prepared and characterised carnuba wax nanostructured lipid carrier containing benzophenone-3 by hot homogenization technique using carnuba wax as the solid lipid and isodecyloleate as liquid oil. The current investigation illustrates the effect of

the composition of the lipid mixture on the entrapment efficiency, in vitro release and stability of benzophenone-3 loaded in these NLCs. A loading capacity of approximately 5% of BZ-3 ($\mu g_{BZ-3}/mg_{lipids}$) was characteristic of these systems.

- **Varsha B. Pokharkar*et al.*, 2011,** developed and optimized Eugenol loaded nanostructured lipid carriers for periodontal delivery by hot homogenization method. High entrapment efficiency was attributed to the imperfections in the crystal lattice leading to increased drug loading. NLC based gel exhibited initial faster onset of release followed by sustained release as evident from comparative study of plain with drug loaded gel.NLC based gel were found to be stable for six months as per ICH guidelines.
- **Yamsani MR *et al* ., 2011,** formulated Domberidone loaded solid lipid nanoparticles and nanostructured lipid carrier by hot homogenization followed by ultrasonication technique using Trimyristin as solid lipid, CetylPalmitate as liquid lipid and a mixture of soyphosphatidylcholine (99%) and Tween 80 as surfactant. The entrapment efficiency and the drug release profile depend on the concentration of lipid and surfactant mixture employed. NLC showed higher entrapment efficiency and faster release profile compared to SLN.
- **Hyun jin Park *et al.*, 2010,** reviewed anti-microbial properties of chitosan and mode of action. Owing to its high biodegradability, non-toxicity and antimicrobial properties,Chitosan-mediated inhibition is affected by several factors can be classified into four types as intrinsic, environmental, microorganism and physical state according to their respective roles. In this review antimicrobial activity in soluble state and solid state are comparatively discussed.

- **Yin-ku Lin *et al.*, 2010,** evaluated the potential of nanostructured lipid carrier (NLCs) loaded with lipophilic Calcipotriol and hydrophilic Methotrexate as topical therapy. NLC composed of Precirol ATO 5 with various amount of squalene as the liquid lipid were prepared. The range of particle size of the NLC preparations was 270 to 320 nm, with vehicles containing a higher Precirol amount exhibiting a larger diameter. NLCs with a higher Precirol/Squalene ratio also showed greater polarity in their molecular environment. The present work confirmed that NLC systems are a promising carrier for the topical delivery of anti-psoriatic drugs as revealed by enhanced skin permission, negligible skin irritation and the compatibility of the two drugs.
- **Rania A. Sanad*et al.*, 2010,** formulated Oxybenzone loaded Nanostructured Lipid Carriers (NLCs) by solvent diffusion method and evaluated using a complete 2^3 factorial design. The study design involves the investigation of the effect of three independent variables namely liquid lipid type (Miglyol 812, Oleic acid), liquid lipid concentration (15% and 30%) and Oxybenzone concentration (5% and 10%) on particle size and entrapment efficiency. NLCs prepared using Miglyol 812, 15% liquid type, 10% Oxybenzone showed lower particle size, higher %EE and slower drug release when compared to those prepared using Oleic acid. The incorporation of Oxybenzone into NLC greatly increased the sun protection factor, erythemal UVA protection factor with the advantage of overcoming the side effects of free Oxybenzone as evidenced by low irritation potential.
- **Ibrahim A. Alsarra*et al.*, 2009,** formulated Chitosan topical gel in the management of burn wounds. The treated wounds were found to be contract at

the highest rate with high molecular weight-high degree of acetylation Chitosan treated rats as compared to untreated and fusidin ointment treated rats. Histological examination and collagenase actively studies revealed advanced granulation tissue formation and epithelisation in wounds treated with high molecular weight Chitosan than medium or low molecular weight Chitosan.

- **KesavanBhaskar *et al.*, 2009,** prepared aqueous dispersions of lipid nanoparticles – Fluriprofen solid lipid nanoparticles and fluriprofennanostuctured lipid carriers by hot homogenization followed by sonication technique and then incorporated into freshly prepared hydrogels for transdermal delivery. FLUNLC showed higher entrapment efficiency and faster release profile in comparison to FLUSLN. Both SLN and NLC dispersions and gels enriched with SLN and NLC possessed a sustained drug release over a period of 24 hour but the sustained effect was more pronounced with the SLN and NLC gel formulations. The pharmacokinetic parameter obtained with transdermal gels indicate that the prolonged elimination half and increased bioavailability of the drug from gels compared with oral administration.
- **Jei Lai *et al.*, 2009,** investigated Glyceryl mono oleate (GMO) / Polaxamer 407 cubic nanoparticles as potential oral drug delivery system to enhance the bioavailability of the water insoluble model drug Simvastatin. Almost complete entrapment with efficiency over 98% was achieved due to the high affinity of Simvastatin to the hydrophobic regions of the cubic phase. Pharmacokinetic profiles in beagle dogs showed sustained plasma levels of Simvastatin for cubic nanoparticles over 12 hour.

- **VandanaPatravale*et al.,* 2008,** designed and evaluated Nanostructured Lipid Carriers for parenteral delivery of artemether (ARM). Nanoject NLC of ARM were formulated by micro emulsion template technique. NLC of ARM offer significant improvement in the anti-malarial activity and duration of action of ARM as compared to the conventional injectable formulation.
- **Amdiacinthia menses do rego*et al.,* 2007,** studied "Simvastatin improves the healing of infected skin wounds of rats". A bacteriological exam of wounds fluid for gram positive and gram negative bacteria, the tecidual expression of TNF-α and IL-1α by immune histochemical technique and histological analysis by HE stain in a group is performed whose open infected skin wounds were treated with topical application of Simvastatinmicroemulsion and a second group wounds treated with saline 0.9%. In comparision, wound tissue from SIM group displayed less leucocyte infiltration and revealed wound infection in only one rat of SIM group, whereas polynominal infection is detected in saline group.
- **Silva.A.C *et al.,* 2007,** devoloped Nanostructured Lipid Carriers based hydrogel formulation for topical delivery of Minoxidil for the treatment of alopecia.Lipid nanoparticles are devoloped using stearic acid as solid lipid, Oleic acid as liquid lipid Polaxamer as Surfactant and the formulation was investigated as drug delivery systems by incorporating into hydrogels by using water soluble polymers.Thedeveloped NLC- based hydrogel formulation was shown to be a promising alternative to the conventional solution of minoxidil, avoiding the risk of undesirable side effects with increased therapeutic efficiency.

- **SoutoE.B.** ***et al.,*** **2006,**carried out a project on "The use of SLN and NLC as topical particulate carriers for imidazole anti-fungal agents". In aqueous NLC dispersions prepared with Tripalmitin and Miglyol approximately 95% of clotrimazole was recovered after one year at room temperature. In the system developed for ketoconazole delivery only 54% of drug was recovered after one year at same temperature. These results emphasise the effect of the nature of the lipid as well as of the drug in chemical stability of system. Samples stored at 4ºc and particularly NLC-Based formulations showed to be more suitable for topical purpose.
- **Uner M.*et al.,* 2005,** studied skin moisturizing effect and skin penetration of AscorbylPalmitate entrapped in Solid Lipid Nanoparticles (SLN) and Nanostuctured Lipid Carrier (NLC) incorporated into hydrogel.SLN and NLC were found to sustain the penetration of AP through excised human skin about ½ and 2/3 times compared to NE (P<.001 and P<0.01), respectively due to the solid state of Witepsol E85 in the lipid phase.
- **Sun Hang cho*et al.*, 2004,** developed selfmicroemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of Simvastatin in beagle dogs. The optimal formulation of SMEDDS containing Simvastatin (high drug loading and small particle size) was as follows :Capryol 90 of 37%, Carbitol of 28% and Cremaphore EL of 28% as oil, surfactant and co-surfactant respectively. In vitro dissolution studies and in vivo studies revealed that significantly greater drug release and higher extend of absorption than the conventional tablet.

CHAPTER – IV

AIM OF THE WORK

CHAPTER IV

AIM OF THE WORK

Chronic wounds develop from slow healing tissue injuries (beyond 12 weeks) which often reoccur. The reasons of healing failure depend on repeated tissue insults or underlying physiological conditions such as diabetes and malignancies, persistent infections, poor primary treatment and other patient related factors. Diabetes mellitus is one of the metabolic disorder that implies normal steps of wound healing process.

Simvastatin belongs to the most effective class of drugs which lowers plasma cholesterol levels by competitive inhibitionof the HMG-CoA reductase, responsible for the first step in the synthesis of sterols. Clinical studies provide growing evidence thatstatins have been shown to have a number of beneficial effects that are not related to lipid lowering. Statins have anticoagulant, immunosuppressive, and antiproliferative effects that could conceivably affect wound healing or the risk of wound complications by increasing both angiogenesis and lymphangiogenesis. Moreover, the safety profile of statins is excellent and the major side effects are rare and mostly reversible.

Topical drug delivery with conventional dosageforms like creams, ointments and moisturizers offer disadvantages viz. difficulty to control and regulate the amount of drug reaching the differentlayers of skin, this problem can be overcome by formulating the drug using acarrier that can enhance the permeation efficiency, increase contact time andare easy and stable upon formulation.Nanostructured Lipid Carriers (NLC) are one of the promisingformulations that can be used for topical delivery of drug; they are used as analternative to liposomes and emulsions as drug delivery system. NLC offeradvantages like, controlled release, drug targeting, penetration enhancementand increased skin hydration, NLC's can also overcome the problemassociated with solid lipid nanoparticles. As they have high loading capacityand there is reduced chances of drug expulsion during storage. Moreoverlarge water content associated with solid lipid nanoparticles creates apotential problem of incorporating them as topical formulations. Since NLCare small particles with size less than 400nm, their ability to cause skinhydration due to occlusion of skin is more, that increases the stratum corneumhydration and thus improves the percutaneous absorption of drug.

The main objective of the project was the development of topical formulation to be applied in treatment of skin wounds. Hydrogels are one of the most popular types of wound dressings, have shown thesuperiority as they can provide a moist environment for the wound and at the sametime deliver the incorporated drug to the woundand hydrogels of natural origin are known to have several advantages(high biodegradability non-toxicity and anti-microbial properties) over synthetic origin hydrogels, Chitosan based hydrogels were selected as delivery system for wound treatment.

The objective of the present study was to develop formulations of NLC based hydrogel for topical delivery of Simvastatin. The prepared gels were evaluated for physical appearance, texure analysis, skin sensitivity test, ex-vivo permeation study, drug deposition study and wound-healing power in allaxon-induced diabetic male albino rats.

CHAPTER – V

PLAN OF WORK

CHAPTER V

PLAN OF WORK

A) STANDARD CURVES FOR SIMVASTATIN:

1. Preparation of calibration medium.
2. Estimation of (absorption maximum) λmax .
3. Preparation of calibration curve using saline phosphate buffer solution pH(7.4) containing 0.15% SDS.

B) COMPATIBILITY STUDIES FOR DRUG AND LIPID:

1. Infrared (IR) spectroscopic studies.
2. Differential scanning Colorimetric (DSC) studies.

C) FORMULATION OF SIMVASTATIN LOADED NANOSTRUCTURED LIPIDCARRIER:

Formulation of simvastatin loaded nanostructured lipid carrier using different lipids (glycerylmonostearate, glyceryl monooleate, glycerylbehenate, propylene glycol mono caprylate, stearic acid and oleic acid) at different drug to lipid ratios (1:3, 1:5 and 1:7) and different stabilizers(Phopolipon 90G) concentration (1%, 2% and 3%) by using hot homogenizationtechnique followed by ultrasonication.

D) CHARACTERIZATION OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIER (NLC):

1. Determination of physical properties.
2. Determination of drug content.
3. Determination of drug entrapment efficiency by centrifugation method.

4. *In vitro* release studies of Simvastatin loaded nanostructured lipid carrier using dialysis membrane.
5. Kinetics of drug release
6. Determination of particle size and zeta potential using Malvern particle size analyser
7. Selection and evaluation of best formulation.
    a. Infrared (IR) Spectroscopic studies.
    b. Morphology of NLC by Scanning electron microscopy (SEM) technique.

E) SIMVASTATIN LOADED NLC BASEDHYDROGEL

Hydrogels(HG) are prepared using Chitosan-4%, followed by the drug & the selected best NLC formulations were incorporated into the HG in order to obtain plain & NLC based hydrogels respectively.

F) EVALUATION OF PREPARED NLC BASED HYDROGEL

1. Determination of Physicochemical properties
2. pH Measurements
3. Drug Content
4. Texture analysis
5. *Ex vivo* skin permeation studies
6. Drug deposition study
7. Ex-vivo Release Kinetics
8. Invivo Diabetic wound healing activity
9. Skin sensitivity Test

CHAPTER –VI

MATERIALS AND EQUIPMENTS

CHAPTER VI

MATERIALS & EQUIPMENTS

MATERIALS USED:

S.NO	MATERIALS	DISTRIBUTORS
1	Simvastatin	Gift sample from Bafna Pharmaceuticals, Chennai.
2	Glyceryl mono stearate	Central drug house, New Delhi.
3	Glyceryl behenate	Orchid Pharma, Chennai.
4	Stearic acid	Central Drug House (P) Ltd, New Delhi
5	Glyceryl mono oleate	Otto chemicals, Mumbai.
6	Propylene glycol mono caprylate	Gift Samples from Gattefosse India Pvt Ltd, Mumbai
7	Oleaic acid	Central Drug House (P) Ltd, New Delhi
8	Phospolipon 90G	Gift sample from phospholipid GmbH, Germany
9	Tween 80	Lobachemie, India
10	Chitosan	Himedia Lab, Mumbai
11	Disodium hydrogen phosphate	Nice chemicals Pvt Ltd, Kerala.
12	Potassium dihydrogen phosphate	High purity laboratory chemicals, Mumbai.
13	Sodium chloride	Central Drug House (P) Ltd, New Delhi.
14	Sodium Dodecyl Sulphate	Rankem Laboratory Reagent, New Delhi
15	Acetonitrile	Central Drug House (P) Ltd, New Delhi

EQUIPMENTS USED:

S.NO	EQUIPMENTS	SUPPLIERS
1	Electronic weighing balance	A & D Company, Japan.
2	UV-Visible spectrophotometer	Shimadzu Corporation, Japan.
3	FT-IR	Shimadzu, Japan.
4	Texture Anayser TA- XT Plus	Stable Microsystems, UK
5	Differential Scanning Calorimetry	DSC Q 200, Mumbai.
6	Mechanical stirrer	Scientific industries, India.
7	Magnetic Stirrer	MC Dalal & co, Chennai.
8	Cooling Centrifuge Apparatus	Eppendorf Centrifuge 5417R, Germany
9	Scanning electron microscope	Hitachi X650, Tokyo, Japan
10	Particle size analyser	Malvern Instrument, U.K.
11	Environmental chamber	Inlab equipments Pvt. Ltd, Madras.
12	Rotary shaker	Secor, India.
13	Refrigerator	Kelvinator, India.

CHAPTER –VII

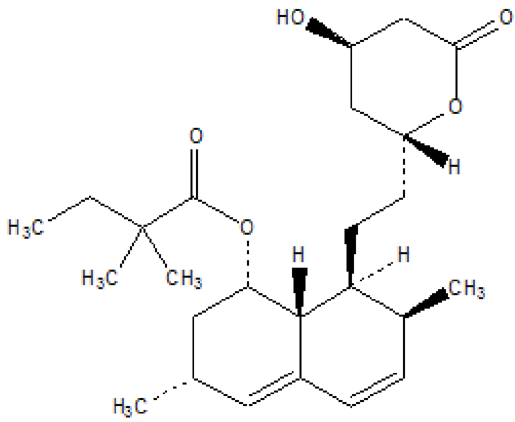
DRUG PROFILE

CHAPTER VII

DRUG PROFILE

SIMVASTATIN

STRUCTURAL FORMULA



SYNONYMS

SYNVINOLIN

VELASTATIN

L-644128-000U

MK 733

EMPIRICAL FORMULA

$C_{25} H_{38} O_5$

CHEMICAL NAME

(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl,2,2-dimethylbutanoate

DESCRIPTION

Nature : White crystalline powder

Molecular weight : 418.6

Solubility : Practically insoluble in water; Freely soluble in alcohol, chloroform and in methanol

partition coefficient (octanol/water) :4.68

MECHANISM OF ACTION

Simvastatin, a cholesterol- lowering agent derived from fungi *Aspergillusterreu*s. When given orally, simvastatin (a lactone) undergoes hydrolysis and is converted to the β-hydroxyacid form by cytochrome-3A, a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase; the enzyme which catalyses the conversion of HMG-CoA to mevalonate, which was an early and rate limiting step in the biosynthesis of cholesterol. The competitive inhibition of the enzyme HMG-CoA reductase promotes two physiological responses in addition to the reduction in plasma cholesterol, increase in the number of low density lipoprotein (LDL) hepatic receptors and immunomodulatory activities. Clinical studies provide growing evidence that statins present anti-inflammatory properties including a reduction of inflammatory mediators such as C-reactive protein, interleukin-6 (IL-6), tumour necrosis factor-a (TNF-a), monocyte chemoattractant protein-1 (MCP-1) and the endothelium-leukocyte interaction.

Simvastatin increases the bioavailability of nitric oxide upregulating NOS and by increasing the stability of NOS through post-transcriptional polyadenylation. It is unclear as to how Simvastatin brings about these effects though they may be due to decreased concentrations of mevalonic acid.

PHARMACOKINETICS

Absorption: Peak plasma concentrations of active plus latent inhibitors (total inhibitors) of simvastatin were attained within 1.3 to 2.4 hours postdose. While the recommended therapeutic dose range is 5 to 40 mg/day, there was no substantial deviation from linearity of AUC of inhibitors in the general circulation with an increase in dose to as high as 120 mg. Relative to the fasting state, the plasma profile of inhibitors was not affected when simvastatin was administered immediately before an American Heart Association recommended low-fat meal. The absolute bioavailability of β-hydroxyacid is 5%.

Distribution: Both simvastatin and it's β-hydroxyacid metabolite are about 98% and 94% bound to plasma proteins respectively.

Metabolism: Simvastatin is administered as an inactive lactone prodrug and has two separate metabolic pathways. Oxidative biotransformation is the primary pathway mediated primarily by cytochrome P450 isoenzyme CYP3A4, and hydrolyzation of simvastatin acid by carboxylesterase is another pathway leading to non-enzymatic metabolism into an active β-hydroxyacid form. The major active metabolites include 3-hydroxy, 3-hydroxy-3-methyl and 3-oxomethylene derivatives and its analogues of 6-hydroxymethyl and 6-carboxylic acid derivatives (these are the biliary metabolites).

Excretion: The drug and its metabolites are primarily excreted in the feces (90%) and about 10 to 15% is recovered in the urine, mainly in inactive forms. The half life of the active β-hydroxyacid metabolite is 1.9 hours and simvastatin is 2 hours.

Age: There were no difference in plasma concentrations of simvastatin between elderly and younger patients.

INDICATIONS AND USAGE

Used as an adjunt to dietary therapy to treat primary hypercholesterolemia (heterozygous familial and nonfamilial) mixed dyslipidemia and hypertriglyceridemia and used to reduce the risk of CHD mortality and cardiovascular events. Also indicated for homozygous familial hypercholesterolemia as an adjunct to other lipid lowering therapies or when other such therapies are not available.

DOSE

5-80 mg orally once daily

DOSAGE FORMS

Tablet-Oral 5mg, 10mg, 20mg, 40mg, 80mg

ADVERSE EFFECTS

Rhabdomyolysis with myoglobinuria and acute renal failure and myopathy (including myolysis).

Upper respiratory infection

Headache

Myalgia

Abdominal pain

Constipation and Nausea

OVERDOSE

There is no specific treatment in the event of overdose. In the event of overdose, thepatient should be treated symptomatically and supportive measures instituted as required. Hemodialysis does not significantly enhance clearance ofclearance of simvastatin (Drug Bank: Simvastatin).

WARNINGS AND PRECAUTIONS

Skeletal muscle effects (e.g., myopathy and rhabdomyolysis)

Risks increase with highest doses and concomitant use of certain medicines like cyclosporine and Danazol. Predisposing factors include advanced age (≥ 65) , female gender, uncontrolled hypothyroidism and renal impairment.

Patients should be advised to promptly report any unexplained and/or persistent muscle pain, tenderness, or weakness. Simvastatin theraphy should be discontinued immediately if myopathy is diagnosed or suspected.

Liver enzymes abnormatilies and monitoring

Persistant elevations in hepatic transaminases can occur. Monitor liver enzymes before and during treatment. (www.fdadrug.info.cfm).

Persistant elevations in hepatic transaminases can occur. Monitor liver enzymes before and during treatment.**(www.fdaDrug.Info.cfm)**

CHAPTER –VIII

EXCIPIENTS PROFILE

CHAPTER VIII

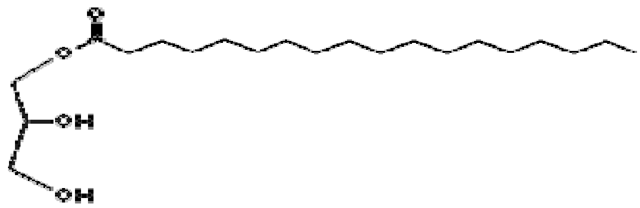
EXCIPIENTS PROFILE

GLYCERYL MONOSTEARATE

SYNONYM:

Glyceryl stearate, Monostearin

STRUCTURE:



CHEMICAL NAME:

3-Stearoyloxy-1,2-propanediol; Octadecanoic acid, 2,3-dihydroxypropyl ester; Stearic acid 1-monoglyceride; 1,2,3-Propanetriol 1-octadecanoyl ester.

EMPIRICAL FORMULA:

$CH_3\ (CH_2)_{16}COOCH_2CHOHCH_2OH$

DESCRIPTION:

Physical state	:	White or cream colored waxy solid.
Melting point	:	63 - 68°C
Boiling point	:	> 100°C
Solubility in water	:	soluble in hot water

Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0
Molecular weight	:	358.56
Functional category	:	Emulsifying agent

STABILITY AND STORAGE CONDITIONS:

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS:

Keep away from heat and ignition.

REGULATORY STATUS :

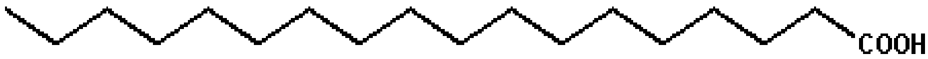
Induced in the FDA inactive ingredients.Recognized by GRAS status.(Handbook of Pharmaceuticals Excipients., 2009, 831-824)

STEARIC ACID

SYNONYMS:

Cetylacetic acid; stereophonic acid; Tegostearic.

STRUCTURE:



CHEMICAL NAME:

Octadecanoic acid

EMPIRICAL FORMULA:

$C_{18}H_{36}O_2$

DESCRIPTION:

Physical state : Crystalline solid/white or yellowish powder.

Melting point : 554°C

Boiling point : 383°C

Solubility : Freely soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.

HLB value : 15

Molecular weight: 284.47

Functional category : Emulsifying agent ,Solubilizing agen, Tablet and capsule lubricant

STABILITY AND STORAGE CONDITIONS:

It is a stable material; an antioxidant may also be added to it. The bulk material should be stored in a well-closed container in a cool, dry place.

SAFETY

It is generally regarded as a nontoxic and nonirritant material. However, consumption of Excessive amounts may be harmful.

HANDLING PRECAUTIONS

Stearic acid dust may be irritant to the skin, eyes, and mucous membranes. Eye protection, gloves, and a dust respirator are recommended. Stearic acid is combustible.

REGULATORY STATUS

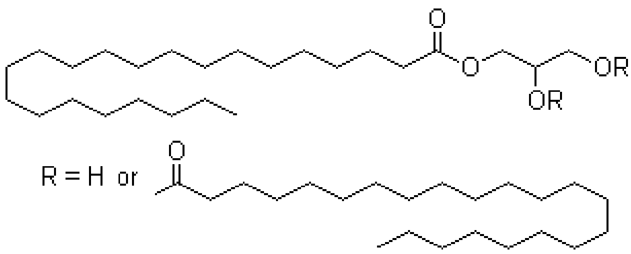
Included in the FDA Inactive Ingredients Guide (sublingual tablets; oral capsules, solutions, suspensions, and tablets; topical and vaginal preparations).

GLYCERYL BEHENATE

SYNONYMS:

Compritol 888 ATO; 2,3-dihydroxypropyl docosanoate; docosanoic acid, glycerylmonobehenate, 1,2,3-Propanetriol docosanoate.

STRUCTURE:



EMPIRICAL FORMULA:

$C_3H_8O_3 \cdot x(C_{22}H_{44}O_2)$

DESCRIPTION:

Physical state : Fine white powder

Melting point : 65–77ºC

Boiling point : 306 ºC

Solubility : Soluble, when heated, in chloroform and dichloromethane, practically insoluble in ethanol (95%), hexane, mineral oil,

HLB value : 12

Molecular weight : 414.66

Functional category : Coating agent, Tablet binder, Tablet and capsule lubricant

STABILITY AND STORAGE CONDITIONS:

It should be stored in a tight container, at a temperature less than 358C.

SAFETY:

It is generally regarded as a relatively nonirritant and nontoxic material.

HANDLING PRECAUTIONS:

It emits acrid smoke and irritating fumes when heated to decomposition.

REGULATORY STATUS :

Included in the FDA Inactive Ingredients Guide (capsules and tablets).(www.sciencelab.com, www.parchem.com, Handbook of Pharmaceuticals Excipients., 2009, 819-824.)

PROPYLENE GLYCOL MONOCAPRYLATE

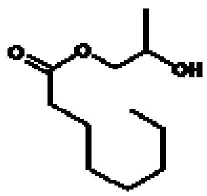
SYNONYMS:

Capryol 90, 1,2propanediolmonocaprylate, Propylene glycol caprylate

EMPIRICAL FORMULA:

$C_{11} H_{22} O_3$

STRUCTURAL FORMULA:



DESCRIPTION:

Appearance	:	oily liquid
Odour	:	Faint
HLB VALUE	:	6
Molecular weight	:	202.29

STORAGE

Stable under ordinary condition.

APPLICATION

It is used as a lipophilic emulsifier and emulsion stabilizer in food and personal care products.(www.chemicalland 21.com)

OLEIC ACID

SYNONYM

Crodolene, Crossential 094, elaic acid glycon, Oeic acid, Priolene.

CHEMICAL NAME

(Z)- 9- octadecenoic acid.

EMPIRICAL FORMULA

$C_{18} H_{34} O_2$

DESCRIPTION

A yellowish to pale brown oily liquid with characteristic of lard like odour

Boiling point : 286°C

Density : 0.895 g/Cm^3

Melting Point : 4°C

Solubility : Miscible with benzene, chloroform,ethanol(95%), ether, hexane, and fixed and volatile oils, practically insoluble in water

Molecular weight : 282.47

FUNCTIONAL CATEGORY

- Used as an emulsifying agent in foods and topical pharmaceutical formulations.
- To improve the bioavailability of poorly water-soluble drugs.
- It is used in oral and topical pharmaceutical formulations as a penetration enhancer.

STABILITY

An exposure to air, gradually absorbs oxygen, darkens in colour, and develops a more pronounced odour. At atmospheric pressure, it decomposes when heated at 80-100°C

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

LD_{50} (rat oral) - 74g/Kg.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

REGULATORY STATUS

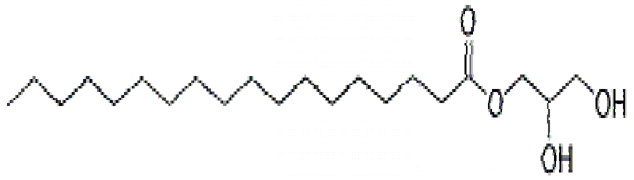
GRAS listed.

GLYCERYL MONOOLEATE

SYNONYM

Glycerylmonooleate, monoolein

STRUCTURE



CHEMICAL NAME

3-Stearoyloxy-1,2-propanediol; 1,2,3-Propanetriol 1-octadecanoyl ester

EMPIRICAL FORMULA

$CH_3\ (CH_2)_{16}COOCH_2CHOHCH_2OH$

DESCRIPTION

Physical state	:	soft solid waxy
Melting point	:	40°C
Boiling point	:	> 100°C
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0
M.W	:	358.56
Functional category	:	Emulsifying agent

STABILITY AND STORAGE CONDITIONS

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

Keep away from heat and sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. (www.sciencelab.com, www.parchem.com,)

PHOSPHATIDYLCHOLINE (PHOSPHOLIPON 90)

CHEMICAL NAME [69]

[(2R)-3-hexadecanoyloxy-2-[(Z)-octadec-9-enoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphat

EMPIRICAL FORMULA

$C_{42}H_{82}NO_8P$

DESCRIPTION

Pale yellow to yellow granular powder

Moisture : maximum 1.5%

Acid value	:	maximum 0.5
Peroxide value	:	0.5
Molecular weight	:	790
Functional category	:	Solubilizer,Emulsifier, Micelle forming agent

STORAGE

Under dry condition, at maximum 8°C sealed under inert gas.Stored in a freezer at -20°C further improves the shelf life and is therefore recommendable.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

APPLICATIONS

Preparation of mixed micelles, liposomes and microemulsions.

Solubilizer for parenteral administration forms

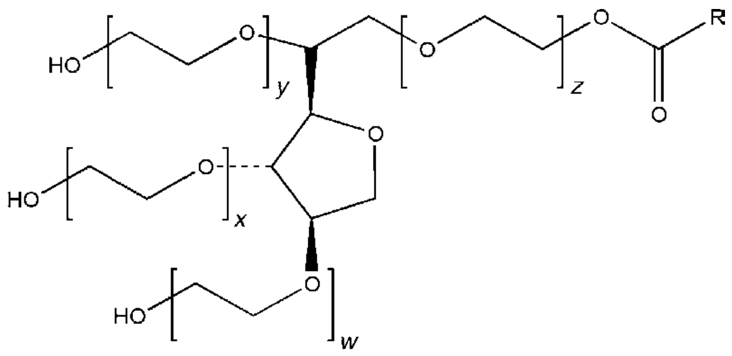
Emulsifier for pharmacy, dermatology and cosmetics

POLYSORBATE 80

SYNONYM

Atlas E, Cappmul POE-O, Glycospere o-20,Tego SMO 80,Tego SMO 80 X, Tween 80.

STRUCTURE



CHEMICAL NAME

(Z) Sorbitan mono-9- Octadecanoate poly (oxy 1,2, ethanediyl) derivatives.

EMPIRICAL FORMULA

$C_{64} H_{124} O_{26}$

DESCRIPTION

Yellow oily liquid.

Molecular weight	:	1310.00
Acid value	:	2.0
Hydroxyl value	:	65 – 80
Saponification value	:	45 - 55
Density (g/cm^3)	:	1.08g/cm^3
HLB Value	:	15
Solubility	:	Soluble in ethanol and water. Insoluble in mineral oil

FUNCTIONAL CATEGORY

Emulsifying agent.Nonionic surfactant, Solubilizing agent, Wetting agent

STABILITY

Gradual soap formation occurs with strong acids or bases

Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight LD_{50} (Mouse, oral)-25g/Kg.

HANDLING PRECAUTIONS:

Eye protection and Gloves are recommended. (Handbook of Pharmaceuticals Excipients., 2009)

CHITOSAN

Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolarization and it is therefore not easily defined in terms of its exact chemical composition. Partial deacetylation of chitin results in the production of chitosan which is a polysaccharide comprising copolymers of glucosamine and n-acetylglucosamine.Chitosan is commercially available in several types and grades that vary in molecular weight between 10,000 and 1,000,000 and vary in degree of acetylation and viscosity.

SYNONYMS

2-amino-2 –deoxy-(1,4)-ß-d-glucopyranan; deacetylated chitin; deacetyl chitin; ß-1,4- amino 2-deoxy-d-glucopyranosamine

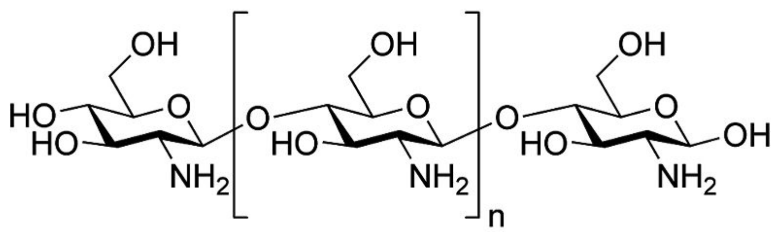
CHEMICAL NAME

poly-ß-(1,4)-2-amino-2-deoxy-d-glucose

DESCRIPTION

chitosan occurs as odorless,white or creamy whitepowder or flakes.fibre formation is quite common during precipitation and the chitosan may look ‘cotton like’.

STRUCTURAL FORMULA



FUNCTIONAL CATEGORY

Coating agent, disintegrant; film forming agent; mucoadhesive; tablet binder; viscosity-increasing agent

TYPICAL PROPERTIES:

Acidity

pH 4.0-6.0(1%w/v aqeous solution)

Density-1.35-1.40 g/cm3

Glass transition temperature-203°C

SOLUBILITY

Sparingly soluble in water, practically, insoluble in ethanol95% and other organic solvents and neutral or alkali solutions at pH above 6.5.

INCOMPATIBILITY

Chitosan is incompatible with strong oxidizing agents.

SAFETY

Chitosan is investigated widely for use as an excipient in oral and other pharmaceutical formulation. It is biocompatible with both healthy and infected skin.chitosan has been shown to be biodegradable.

STABILITY AND STORAGE CONDITIONS:

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in tightly closed comtainer in a cool, dry place and it should be stored at a temperature of 2-80c

CHAPTER – IX

EXPERIMENTAL DETAILS

CHAPTER IX

EXPERIMENTAL PROTOCOL

A) STANDARD CURVES FOR SIMVASTATIN

1. Preparation of calibration medium: (U.SPharmacopoeia ., 2007)

Phosphate buffer saline (PBS) pH 7.4containing 0.15% sodium dodecyl sulfateis prepared by dissolving 1.5 g of sodium dodecyl sulfate,2.38g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 g of sodium chloride in 1000 ml of distilled water. The pHis adjusted if necessary.

2. Determination of (absorption maximum) λmax:

A stock solution is prepared by dissolving an approximate quantity (100 mg) of pure drug in 100 ml Acetonitrile (1000 µg/ml). The concentration of stock solution is further diluted with phosphate buffer solution (PBS) pH 7.4 to form secondary solution resulting in the concentration of 100 µg/ml and to prepare solution with 10 µg/ml concentration and scanned at different wavelength range of 200 – 400 nm in an UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan). From the absorbance of the resulting solution λmax is determined.

3. Preparation of calibration curve using phosphate buffer solution (PBS) pH 7.4 containing 0.15 % SDS:

From the above prepared secondary stock solution2ml, 4ml, 6ml, 8ml, 10ml, 12ml, 14ml 16ml, 18ml, 20ml samples are pipetted into 100 ml volumetric standard flasks separately and made up to the volume with Phosphate buffersaline pH 7.4 containing 0.15% SDS to get concentrations of 2 µg/ml, 4 µg/ml,6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, 20 µg/ml of drug solutions respectively. The absorbance of the solutions is measured by using Ultraviolet visible

(UV) spectrophotometer (Shimadzu UV-1700 pharma spec, Japan) at λmax 239 nm. Calibration curve is plotted by using concentration in x – axis and absorbance in y – axis(Singla. N *et al*.,2009).

B) COMPATIBILITY STUDIES FOR DRUG AND LIPID:

For the development of formulation dosage form, preformulation studies is carried out to confirm no interaction exist between the drug and excipients. It gives information needed for selection of excipients with the drug for the formulation of nanodispersion. Infrared spectrophotomeris used to check the compatibility studies between lipids (glycerylmonostearateglycerylbehenate, stearic acid, glycerylmonooleate, capryol, & oleic acid) and drug.

1. Infrared (IR)Spectroscopic studies:

The spectroscopic studies are carried out to find the interaction between pure drug, lipids and surfactants and its physical mixture by KBr pellet technique using FTIR spectrophotometer (shimadzu, RX 1, Japan). The IR spectrum of the best formulation is then compared with the spectrum of pure drug (Simvastatin) to assess the compatibility of the excipients and drug. The scanning range is 450-4000 cm^{-1} and the resolution is $4cm^{-1}$(Senthilnathan .B *et al*.,2011, Priyanka& Abdul HasanSathali A., 2012).

C) FORMULATION OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIER:

Simvastatinloaded nanostructured lipid carrier is prepared by modified method of**hot homogenization** followed by ultrasonication.

Briefly, thelipophilic and hydrophilic surfactants were added into the lipid phase containing the solid lipid and liquid lipid (70:30). The lipidphase is melted at approximately 5 - 10°C above its melting point to avoid recrystallisation of the lipid phase. The volume of water was halved. The volume of the first water potion was adjusted to have the same volume as the lipid phase. The volume of the second water portion was not adjusted.

The first portion of the water was heated to the same temperature of lipid mixture to prevent recrystallisation of lipid phas. Then 1% w/w of simvastatin was added into the melted lipid phase followed by the addition of first hot-water portion immediately into the lipid phase . The mixture of drug, lipid phase, and the water phase was homogenized at 2000 rpm by using mechanical stirrer for 60 min.

During the homogenization process, the second water portion (25º) was added slowly to the mixture to form the pre-emulsion. Then the pre-emulsion is allowed to cool to room temperature and stirred at 400 rpm for 30 min.

Then it is ultrasonicated using a probesonicator processor for 10 min. Finally, the NLC dispersion was cooled rapidly in an ice bath to 25°C (Loo Chew Hung *et al.*, 2011)

D) CHARACTERIZATION OF SIMVASTATIN LOADED NANOSTUCTURED LIPID CARRIER (NLC):

All the formulated Simvastatinloaded nanostructured lipid carrier are evaluated for its drug content, entrapment efficiency, particle size, polydispersity index, zeta potential, *in vitro* drug release and kinetics of drug release.

1. Determination of Physicochemical properties:

The NLC dispersion is to characterize for physicochemical properties such as color, odor and stability after centrifugation (2000 rpm for 30 minutes).

2. Determination of drug content:

The total drug content of NLC dispersion is determined by spectrophotometric analysis. One milligram equivalent of Simvastatin loadedNLC dispersion is dissolved in (1 ml) ofmethonol and is suitably diluted withpH 7.4 saline phosphate buffer to make 10 µg/ ml concentration. The absorbance is measured at 239 nm (λmax) using UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan). From the absorbance the drug content(Premanand .A *et al.,* 2012, Senthilnathan .B *et al.*,2011) is calculated using the formula given below,

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

3. Determination of drug entrapment efficiency:

Simvastatin loaded NLCformulations (1 ml) are centrifuged at 14,000 rpm for 90 minutes at 4°C using a refrigerated centrifuge (Eppendorf, 5417R, Germany) in order toseparate the amount of Simvastatin encapsulated in the NLC from the unentraped drug.The free drug concentration in supernatant layer after centrifugation is determined at λmax (239 nm) using UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan)

The % entrapment efficiency is calculated by the following formula:

$$\%EE = \frac{\text{Total amount of drug taken - Unentraped drug}}{\text{Total amount of drug taken}} \times 100.$$

(Gambhire M.S *et al.,* 2011, Priyanka& Abdul HasanSathali A *et al.*, 2012,Dilip Patel *et al.,* 2012)

4. *In vitro* release studies:

In vitro release of Simvastatin from Simvastatin loadednanostructured lipid carrier formulation is determined by using dialysis bag diffusion technique using pH 7.4 saline phosphate buffer solution as dissolution medium.

Dialysis membrane with pore size of 2.4 nm and molecular weight cut off between 8000 – 13,000 Da is used which could retain NLCs and allow the diffusion of free drug into the dissolution medium. The bags are soaked in distilled water for 24 h before use. The nanosuspension equivalent to (10 mg) of Simvastatin(approximately 0.85 ml) is placed in dialysis bag and sealed at both ends. The dialysis bag is immersed in receptor compartment containing 100 ml of pH 7.4 phosphate buffer solutions in 250 ml beaker maintaining at 37°C ± 2°C and magnetically stirred at 100 rpm. Samples withdrawn at predetermined time intervals of 30 min for first 2hrs and every 60 min for 10 hrs. Sink condition is maintained by replacing with fresh phosphate buffer solution. The content of Simvastatin in the samples is determined by UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan) at 239 nm. All the operations are carried out in triplicate and the average values were taken (Medha Joshi*et al*., 2008, Nisha .N & Abdul HasanSathali.A *et al*., 2012, Premanand .A*et al*., 2012, Gambhire M.S *et al.,* 2011).

5. Kinetics of drug release

In order to investigate the drug release mechanism from NLC formulations, the percentagecumulative drug release data is analyzed with following mathematical model.

- Zero-order
- First order
- Higuchi
- Hixson-Crowell cube root law
- Korsmeyer-peppas model.

• **The zero order rate Equation** describes the systems where the drug release rate is independent of its concentration.

$$Q_t = Q_0 + K_0t$$

Where,Q_t is the amount of drug dissolved in time t,Q_0 is the initial amount of drug in the solution (mosttimes, $Q_0= 0$) and K_0 is the zero order release constantexpressed in units of concentration/time.To study the release kinetics, data obtainedfrom *in vitro* drug release studies are plotted ascumulative amount of drug released *versus* time

•**The first order Equation** describes the release from a system where the release rateis concentration dependent.

$$\log C = \log C_0 – k_t / 2.303$$

Where C is the concentration of the drug at time (t), C_0 is the initial concentration of the drugand k is the first-order release rate constant.Thedata obtained are plotted as log cumulative percentageof drug remaining *vs*. time.

• **Higuchi** described the release of drugs from porous, insoluble matrix as a square root oftime dependent process based on Fickian diffusion as shown in following Equation.

$$Q = kt^{1/2}$$

Where Q is the amount of drug released in time t.This model is based on the hypotheses that (i) initialdrug concentration in the matrix is much higher thandrug solubility; (ii) drug diffusion takes place onlyin one dimension (edge effect must be negligible);(iii) drug particles are much smaller than systemthickness; (iv) matrix swelling and dissolution arenegligible; (v) drug diffusivity is constant; and (vi)perfect sink conditions are always attained in therelease environment.The data obtained are plotted as cumulative percentagedrug release *versus* square root of time

•**Hixson and Crowell** (1931) recognized that the Particles' regular area is proportional to the cuberoot of its volume. They derived the equation

$$\mathbf{W_0^{1/3} - W_t^{1/3} = \kappa\, t}$$

Where W_0 is the initial amount of drug in the pharmaceuticaldosage form, W_t is the remaining amountof drug in the pharmaceutical dosage form at time tand κ (kappa) is a constant incorporating the surface-volumerelation.The equation describes therelease from systems where there is a change in surfacearea and diameter of particles. Tostudy the release kinetics, data obtained from *in vitro* drug release studies are plotted as cube root ofdrug % remaining in matrix *vs*time.

•**Korsmeyer – Peppas model** describes the fractionof drug release relates exponentially with respect to time.

$$\mathbf{M_t / M_\infty = Kt^n}$$

Where M_t / $M_\infty$is a fraction of drug released at timet, k is the release rate constant and n is the release exponent.In this model, the value of *n* characterizes therelease mechanism of drug. To study the release kinetics,data obtained from *in vitro* drug release studies areplotted as log cumulative percentage drug release*versus*log time

Release exponent (n)	**Drug transport mechanism**	**Rate as a function of time**
0.5	Fickian diffusion	$t^{-0.5}$
0.45 < n = 0.89	Non – Fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super case II transport	t^{n-1}

(Suvakanta Dash *et al*., 2010)

6. Particle size and zeta potential:

Particle size (z- average diameter), polydispersity index (as a measure of the width of the particle size distribution) and zeta potential(to characterize surface charge of particle and physical stability of colloidal dispersions) of Simvastatin loaded NLC dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments, UK) at 25°C.

Prior to measurements all samples are diluted using ultra – purified water to yield a suitable scattering intensity. The diluted nanoparticle dispersion is poured into the disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analysed. Air bubbles, if any is removed from the capillary before measurement. All measurements is performed in triplicate.(MakarandGambhire*et al*., 2011, PornthidaRiangjanapatee*et al*., 2012).

7. Selection and evaluation of best formulation

The best formulation selection based on the results obtained from particle size, entrapment efficiency, *in vitro* release studies and kinetics of drug release.

a. Infrared (IR)spectroscopic studies:

Infrared spectrum analysis is carried out to find out the interactions between the drug and excipients used as per the procedure mentioned on drug and lipid interaction studies.

b. Morphology of NLC by Scanning electron microscopy (SEM) technique:

Scanning electron microscopy is an excellent tool for physical observation of morphological features of particle both initially and degradation process. It is helpful to examine particle shape and surface characteristics such as surface area and bulk density. The formulations are poured in a circular aluminum stubs using double adhesive tape, and coated with gold in HUS – 5GB vaccum evaporator and observed in Hitachi S – 3000N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X.

E. FORMULATION OF SIMVASTATIN LOADED NLC BASED HYDROGEL

On the basis of particle size, entrapment efficiency and in-vitro release studies, F15 is selected for the preparation of topical gel system.

For the preparation of blank HG, Chitosan was dispersed in 1 % acetic acid solution and subsequently, Glycerine 1.5 ml was added to the aqueous solution,. The mixture was stirred manually for 10 minutes and sonicated to remove air bubbles. The formed hydrogel was left equilibrating for 48 hours in a sealed container at room temperature. Alasarra (2009) and Cao *et al.*(2009). Pure drug and NLC dispersionswere incorporated into the blank HGs, using a high speed stirrerat 1000 rpm for1 min, in a concentration of 50% of the dispersion in the gel to yield plain gel and NLC based HG respectively (Ibrahim A.Alsarra ., 2009, Silva A.C *et al.,* 2012

F. EVALUATION OF THE PREPARED SIMVASTATIN NLC BASED HYDROGEL:

1.Determination of Physicochemical properties:

The hydrogels were characterized for physicochemical properties such as appearance, pH, texture analysis and stability.

2.pH measurements

The pH of the gel formulations are determined by dissolving 1g of gelin 10 ml of distilled water using a pH meter. Measurements are performed at 1^{st,} 15thand 30th day after preparation to detect any pH fluctuations (ShindeAnilkumar .J *et al.,* 2011)

3.Drug content

One gram of gel formulation which contains approximately 5mg of drug is dissolved in (10 ml) of methanol. The resultant mixture is filtered through membrane filter (pore size 0.45 mm) and is suitably diluted with pH 7.4 saline phosphate buffer containing 0.15% SDS to make 10 μg/ ml concentration. The absorbance is measured at 239 nm (λmax) using UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan) (GajananShinde*et al.,* 2013).

4.Texture Analysis

The viscosity of topical NLC based gel formulation is animportant factor in determining residence time of the drug in the skin. The viscosity determination is carried out by Texture Anayser TA- XT Plus (Stable Microsystems, UK) with the accessory Back extrusion cell, with 35mm disc and extension bar using 50 kg load cell

TA Settings:	*Mode*:	Measure Force in Compression
	Option:	Return to Start
	Pre-Test Speed:	1.5 mm/s
	Test Speed:	2.0 mm/s
	Post-Test Speed:	2.0 mm/s
	Distance:	20mm
	Trigger Type:	Auto - 10g
	Tare Mode:	Auto
	Data Acquisition Rate:	250pps

Method : Tests were carried out in a standard size back extrusion container (50mm diameter), approximately 75% full, immediately after removal from storage at a specific temperature e.g. 25C. The extrusion disc (35 mm) was positioned centrally over the sample container. For comparison of cohesiveness and 'work of cohesion' the probe was returned to the same position above the samples after each test. The averages of three readings are used to calculate the viscosities of formulations(Autio, K *et al.,* 2003)

When a 10g surface trigger is attained (i.e. the point at which the disc's lower surface is in full contact with the product) the disc proceeds to penetrate to a depth of 20mm. At this point (most likely to be the maximum force), the probe returns to its original position. The 'peak' or maximum force is taken as a measurement of firmness - the higher the value the firmer is the sample. The area of the curve up to this point is taken as a measurement of consistency - the higher the value the thicker the consistency of the sample.

The negative region of the graph, produced on probe return, is as a result of the weight of sample which is lifted primarily on the upper surface of the disc on return, i.e. due to back extrusion and hence gives again an indication of consistency/resistance to flow off the disc. The maximum -ve force is taken as an indication of the cohesiveness of the sample - the more negative the value the more 'cohesive' is the sample. The area of the negative region of the curve may be referred to as the 'work of cohesion' - the higher the value the more resistant to withdrawal the sample is which is an indication of the cohesiveness and also consistency/viscosity of the sample.

5.*Ex vivo* skin permeation studies

Ex vivo skin permeation of of Simvastatin NLC based Hydrogelare studied using locally fabricated Franz diffusion cell with an effective permeation area and the receptor cell volume 2.54cm^2 and 15ml respectively.

Rats (male albino) 6 to 8 weeks old, weighing 150 to 200g are sacrificed for abdominal skin. After removing the hair the abdominal skin is separated from the underlying connective tissue with scalpel. The excised skin is placed on aluminium foil and the dermal side of the skin is gently teased off for any adhering fat and/or subcutaneous tissue. The skin is checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or cervices in the portion that is used for transdermal permeation studies. The *ex vivo*study was approved by the institutional ethical committee.

The skin is mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 is filled in the receptor compartment. The NLC based gel formulation is applied on the skin in donor compartment which is then covered with aluminium foil to avoid any evaporation process. Sample is withdrawn through the sampling port of diffusion cell at predetermined time intervals for 12 hours, diluted to 10µg/ml concentration with phosphate buffer saline pH 7.4 and analyzed for drug content by UV-Visible spectrophotometer at λ_{max}. The receptor medium is immediately replenished with equal volume of fresh medium.

Similar experiments are performed for Simvastatin plain gel. Experiments are conducted in triplicate. Sink condition is maintained for all the experiments. The percentage of drug release is plotted against time to find the drug release pattern (Gajanan S Sanap*et al.,* 2013,Dilip Patel *et al.,* 2012)

6.Drug deposition study:

After 24 hrs release study, drug remained on skin was determined by scraping and then washing skin 3-4 times with diffusion medium and mixed. These washing were filtered through 0.45µ filter. This sample was analyzed by diluting with medium to make 10 µg/ ml and absorbance was measured at 239 nm using UV-VIS spectrophotometer (Shimadzu UV-1700 pharma spec, Japan). The same procedure was performed for NLC Based HG & plain HG formulations. At the end of 24 h, drug deposited in skin was also determined. A skin was cut into small pieces, homogenized and extracted with methanol and suitably diluted using pH 7.4 saline phosphate buffer and analyzed spectrophotometrically at 239nm.

7. *Ex vivo* release kinetics

The procedure for *ex vivo* release kinetics is the same as *in vitro* release kinetics study and is already discussed.

8.Invivo Diabetic Wound healing activity

The study was approved by the institutional animal ethics committee of KM college of pharmacy, Madurai with proposal number of IAEC/125/KMCP/261220708/2013-2014 which is constituted as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals) guidelines. The studies are conducted on albino rats of either sex, weighing 200 ± 20 g. are selected Over Night fasted rats were made Diabetic by Single injection of Allaxon. Seven days later, rats with blood glucose levels between 300-400mg/dl were selected for invivo evaluation. The selected animals were divided into 3 groups with 6 animals in each group. The first was a control group that is treated with NLC based gel without drug. The other was the test groups and received the prepared gels.

The back of the mouse was shaved and then sterilized using an alcohol swab. Wounds of approximately $2cm^2$ in surface area and 2mm in depth were made with sterile scalped on the shaved area of the back skin below the shoulder blades under light ether anesthesia. A wound placed in this area cannot be reached by the animal and therefore prevent self-licking. The wounding day was considered as day zero. The wound area treated with topical application of prepared gel (100mg once daily) till the wounds were completely healed.

A measurement of the healed wound area as well as that unhealed was carried out by tracing the wound areas on butter paper and was replicated on a graph paper, from that the areas were measured bysquare counting procedure. The number of squares (0.01cm2) that appear completely (Nc) and partially (Np) inside thetracing were counted and area was determined using the followingformula: Ac+p= (Nc+0.4xNp) x 0.01.The wounds are monitored and the area of wound was monitored from the wounding day followed by 4,7,10 & 12th day. The percentage of wound contraction is calculated as the following.

$$\% \text{ of wound contraction} = \frac{\text{Wound area on day (zero)} - \text{Wound area in day (n)}}{\text{Wound area on day (zero)}} \times 100$$

GROUP-1(STANDARD GROUP)

Animal were treated with NLC based hydrogel without drug

GROUP-2(STANDARD GROUP)

Animals were treated with Simvastatin plain gel

GROUP-3(TEST GROUP)

Animals were treated with gel Simvastatin NLC based Hydrogel

(UsamaFarghalyAly., 2012, Ibrahim A. Alsarra., 2009, Schubert V., 1997)

9. Skin Sensitivity Test :

Rats (200 -250 g) of either sex used for testing of skin irritation. The animals were maintained on standard animal feed and had free of water. The animals were kept under standard conditions. Hair was shaved from back and area of 4 cm^2 was marked on both the sides, one side served as control while the other side was test. Gel was applied (500 mg/animal) twice a day for 7 days and the site was observed for any sensitivity and the reaction if any, was graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but cofluent or moderate but patchy erythema and severe erythema with or without edema, respectively(UsamaFarghalyAly., 2012,GajananShinde*et al.,* 2013).

CHAPTER – X

RESULTS AND DISCUSSION

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A) STANDARD CURVES FOR SIMVASTATIN

1. Preparation of calibration medium

The calibration medium of phosphate buffer saline PBS pH 7.4(with 0.15% SDS) was prepared as per Indian Pharmacopoeia., 2010.

2. Determination of (absorption maximum) λmax

The absorption maximum (λmax) of the Simvastatin was estimated by scanning the drug solution (10 µg/ml) between 200-400 nm regions in the UV spectrophotometer. The obtained spectrum showed that the absorption maximum (λmax) at 239 nm in PBS pH 7.4 (with 0.15% SDS). The absorbance spectra was shown in Figure 1A.

3.Preparation of calibration curve using phosphate buffer (PBS) pH7.4 with 0.15% SDS

The standard calibration curve of Simvastatin was prepared by measuring the absorbance of drug at different concentrations (2-20 µg/ml) using PBS pH 7.4 (with 0.15% SDS). The absorbance of solution was measured at λ_{max} of 239nm.Simvastatin obeys the beer's law within the concentration range of 2-20 µg/ml.From the calibration curve the correlation coefficient was found to be 0.99961. The calibration readings were shown in Table 1 and calibration plot of Simvastatinwas shown in Figure 1B (Singla N et al 2009).

B) COMPATIBILITY STUDIES FOR DRUG AND LIPID

1. Infrared (IR) Spectroscopic studies

IR spectroscopy was used to investigate the interactions between lipid, drug and other excipients. The results of IR Spectra of pure drug, lipidic excipient and physical mixture of lipid and drug were shown in the Figure 2A- 2I.The FT-IR spectral analysis of Simvastatin alone showed that the principal peaks were observed at wave numbers 3551.07, 3011.95, 1725.38, and 1654.01. 1455.34, 1390.72, and 841.96cm-1 (Figure 2A) confirming the purity of the drug

S.NO	FUNCTIONAL GROUPS	OBTAINED WAVE NUMBER
1	Saturated Ketones (C=O)	1725.38 cm^{-1}
2	C=C stretching	1455.34 cm^{-1}
3	$C\text{-}(CH_3)_2$	1390.72 cm^{-1}
4	β – Ketonic ester (COO)	1654.01 cm^{-1}
5	C=CH	3011.07 cm^{-1}
6	Aromatic C=O	1697.41 cm^{-1}
7.	Benzene Ring	1466.91 cm^{-1}

In the FT-IR spectra of the physical mixture of simvastatin, lipids, surfactant and chitosan, the major peaks of Simvastatin were observed at wave numbers 3551.07, 3011.95, 1454.38, and 1725 cm-1. However, some additional peaks were observed with the physical mixture, possibly because of the presence of lipids. (Rahul Nair *et al.*, 2011).It was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug and the physical mixture. Hence the drug and lipid are compatible with each other.

C) FORMULATION OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIERS (NLCs)

The Simvastatin loaded NLC were prepared by hot homogenization followed by ultra sonication method.The initial location of the surfactants greatly influences the properties of the emulsion.A double emulsion is formed during the emulsification process by initially placing the surfactants in the lipid Phase and yielded a very good emulsion with uniform particle size distribution (Lin *et al*., 1975). In addition to the high-temperature effect, the cooling of the pre-emulsion after homogenisation contributes to the narrow particle size distribution for the pre-emulsion which agrees with the finding of Marie P *et al*., 2002

Previouslyit was reported that the improved drug entrapment efficiency and an increased stabilitywith mixure of solid lipids & liquid lipids in the ratio of 70:30.So the NLC composed of lipids at different concentration (3%,5% and 7%) and different percentage of lipid phase surfactant (Phospholipon 90G -1%,2% and 3%) were prepared. The compositions of the formulations are shown in Table 2A-2Cresulted a stable, uniform and homogenous dispersion of NLCs.

i) Impact of lipidson NLC formulations

The excipients selected were needed to be pharmaceutically acceptable, nonirritating, and nonsensitizing to the skin and to fall into the GRAS (generally regarded as safe) category for the formulation of NLCs. Selecting matrix lipids and stabilizers based on solubility can help formulating NLC with good storage stability and a high E.E., Blending a solid lipid with a liquid lipid leads to a less ordered solid lipid matrix providing the possibility for a high drug payload (Hu *et al*., 2006; Muller *et al*., 2002).A range of solid lipids (stearic acid, Glyceryl mono stearate and compritol) , liquid lipids (capryol, oleic acid andGMO) and surfactants (Tween 80) were selected which had the highest solubility of Simvastatin in previously repoted research works (HanaaMohmoud*et al*., 2013; Jie Lai *et al*., 2009;

ii) Impact of surfactant on NLC formulations

The amount emulsifier should be optimum to coverthe surface of the nanoparticles and the right blend of low and high hydrophilic lipophilic balance surfactants leads to the formation of a stable nanoemulsion formulation. (Prachi B. Shekhawat., 2013). Among the various surfactants evaluated, the maximum solubility of Simvastatin was found in Tween 80 so it was selected as aqueous phase surfactant(SarfarazAlam Md.*et al.,* 2012) and the addition of phospholipid reduces the possibility of drug loss to the external phase, and provides more space to incorporate the drug, resulting in improved entrapment efficiency. This may also be due to an excess of phospholipid possibly forming multilayers around the particle (Shah KA et al.,2007).So the impact of Phospholipon 90G on EE at various concentration was investigated by using Phospholipon 90G as lipophilic surfactant.

D) CHARACTERIZATION OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIER (NLC)

1. Determination of Physical properties

The NLC dispersion was milky white in appearance, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 minutes).

2. Determination of drug content

The percentage drug content for all the formulations (F1-F27) shown in the Table 3. The drug content was found in the range of 92.87% - 94.74% indicating uniform distribution of drug in the formulation.

3. Determination of drug entrapment efficiency (EE)

In order to attain optimal encapsulation efficiency, several factors were varied including the type and concentration of the lipid and concentration of lipid phase surfactant material used. In all the formulations, the impact of lipid and surfactant concentration was significant. The results of EE were shown in the Table 4A-4C and Figure 3.

The EE of the formulations F1-F3 (glycerylmonostearate&capryolat 3% and phospholipon 90G at 1% - 3% as surfactant) showed 29.38% - 41.26%; for the formulations F4-F6 (glycerylmonostearate&capryol at 5% and phospholipon 90G at 1% - 3% as surfactant) showed 33.43% - 43.14% and for the formulations F7-F9 (glycerylmonostearate&capryol at 7% and phospholipon 90G at 1% - 3% as surfactant) showed 40.74% - 54.13%.

The EE ofthe formulations F10-F12 (stearic acid & oleic acid at 3% and phospholipon 90G at 1% - 3% as surfactant) showed 25.61% - 41.87%; for the formulations F13-F15 (stearic acid & oleic acid at 5% and phospholipon 90G at 1% - 3% as surfactant) showed 29.18% - 40.64%, andfor the formulations F16-F18 (stearic acid & oleic acid at 7% and phospholipon 90G at 1% - 3% as surfactant) showed 37.77% - 49.72%

The EE of the formulations F19-F21 (compritol and GMO at 3% and phospholipon 90G at 1% - 3% as surfactant) showed 41.98% - 52.84%, forthe formulations F22-F24 (compritol and GMO at 5% and phospholipon 90G at 1% - 3% as surfactant) showed 52.12 - 62.16% (F22-F24) at 5% and forthe formulations F25-F27 (compritol and GMO at 7% and phospholipon 90G at 1% - 3% as surfactant) showed 56.05 - 67.76%.

The influence of surfactant and lipid concentration discussed below.

i) Influence of surfactant on entrapment efficiency

The effect of lipid phase surfactant (Phospholipon 90G) on the entrapment efficiency of Simvastatin loaded Nanostructured lipid carrier were investigated. The results were shown in Table 4A-4C.

From the results it was observed that the influence of surfactant was more pronounced on entrapment efficiency apart from particle size from the production process, the physical long-term stability during storage and drug release profiles.

Among the formulations F1-F9 (GMS &capryol as lipids) the maximum entrapment efficiency was obtained for the formulations F3, F6 and F9 and it was found to be41.26%, 43.14% and 54.13% at 3%, 5% and 7% of lipids respectively.

Among the formulations F10-F18 (Stearic acid & oleic acid as lipids) the maximum entrapment efficiency was obtained for the formulations F12, F15and F18 and it was found to be41.87%, 40.64% and 49.72% at3%, 5% and 7% of lipids respectively

Among the formulations F19-F27 (compritol& GMO as lipids) the maximum entrapment efficiency was obtained for the formulations F21, F24 and F27the EE was found to be 52.84% , 62.16% and 67.76at 3%, 5% and 7% of lipids respectively, while remaining batches showed less entrapment efficiency.

From the prepared NLC formulations, it was observed that an increase in Phospholipon 90G concentration increases EEand the formulations containing Phospholipon 90G at 3% showed higher entrapment irrespective of the lipids composition.

ii) Effect of lipid material on entrapment efficiency

It could be noted that thelipid concentrationalso influenced the entrapment efficiency of NLC. It was based on the length of carbon chain atom and melting point of lipids.

The EE of the formulations F 1- F 9 containing GMS&capryolranging from 29.38 - 54.13%, for the formulations F 10- F 18 containingstearic acid & oleic acid ranging from 25.61- 49.72 % and for the formulationsF 19- F 27 containing compritol ATO 888 & GMO ranging from 41.98 – 67.76 %.

The results indicated that, increasing the lipid concentration also increases the EE. This may be due to the reason that the higher concentration of lipid would provide more space to increment of the lipid content and also reduces the escaping of drug into the external phase thus ensuring highest EE.

The EE of the three lipids was in the order of

Compritol>Glycerylmonostearate> Stearic acid.

Among the lipids used, compritol showed the highest EE when compared to glycerylmonostearate and stearic acid. This may be due to the fact that the presence of long chain fatty alcohol could lead to the creation of a less ordered solid lipid matrix and leaves enough space to accommodate drug molecules(Compritol molecular formula (MF): $C_{25}H_{52}O_6$, glycerylmonostearate MF: $C_{21}H_{42}O_4$ and stearic acid MF: $C_{18}H_{36}O_2$) (Priyanka and Sathali., 2012).

4. *In vitro* release studies

From the results, it was observed that simvastatin loaded Nanostructured lipid carrier containing three different lipids (GMS, Stearic acid and compritol ATO 888) displayed a biphasic drug release pattern with a burst release within 2 hours followed by sustained release. The values are shown in Table 5A-7C and Figure 23A-23C.

Initial burst effect

The initial burst effect of the formulations containing glyceryl mono stearate &capryolvaried from 39.63% – 30.58 % (F1-F3) at 3%; 34.69% – 29.18% (F4-F6) at 5% and 30.73% – 26.01% (F7-F9) at 7% using phospholipon 90G at 1% - 3% as surfactant.

The initial burst effect of the formulations containing stearic acid& oleic acid varied from 43.16% – 31.59 % (F10-F2) at 3%; 38.09% – 32.21% (F13-F15) at 5% and 32.63% – 26.32% (F16-F18) at 7% using phospholipon 90G 1% - 3% as surfactant.

The initial burst effect of the formulations containing compritol and GMO varied from 30.03% – 25.69 % (F19-F21) at 3%; 27.69% – 23.46% (F22-F24) at 5% and 25.85% – 21.68% (F25-F27) at 7% using phospholipon 90G 1% - 3% as surfactant.

The initial burst release might be due to the presence of unentrapped drug in the NLC dispersion. Another reason might be due to most of the liquid lipid being located in the outer shell of the nanoparticles, the oleic acid-enriched outer layers possessed a soft and considerably higher solubility for lipophilic drugs, which ultimately increased the loading of the drug which lead to a drug-enriched shell and could be easily released by diffusion or matrix erosion that is related to burst release at the initial stage (Hu QF et al 2005) .

The other possible explanation for the observed burst release is the large surface area of Simvastatin-loaded NLC as well as the short diffusion distance for Simvastatin from the particle matrix into the dissolution medium since the liquid lipid adheres to the lipid matrix which decreases the diffusion path length of the lipid matrix.(Müller et al., 2002b; Souto et al., 2004; zurMühlen et al., 1998).

From the results higher lipid ratios and Phospholipon 90G - 3% as surfactant containing formulations showed lower release from the lipid due to higher entrapment efficient.

Sustained effect

After the 2 hours burst release, NLC formulations showed sustained release at 12 hours. The release of drug from the formulations containing glycerylmonostearate&capryol as lipids varied from 75.81%-67.84% at 3% (F1-F3); 71.99%-64.72% at 5% (F4-F6) and 68.62%-59.79% (F7-F9) using phospholipon 90G at 1% - 3% as surfactant.

The release of drug from the formulations containing stearic acid& oleic acid as lipidsvaried from 80.35%-69.83% at 3% (F10-F12); 77.25%-70.85% at 5% (F13-F15) and 72.28%-63.63.64% (F7-F9) using phospholipon 90G at 1% - 3% as surfactant.

The release of drug from the formulations containing compritol& GMO as lipids varied from 67.82%-61.29% at 3% (F19-F21); 62.49%-56.61% at 5% (F22-F24) and 60.63%-53.81% (F7-F9) using phospholipon 90G at 1% - 3% as surfactant.

Among the three lipids used, compritol ATO 888 showed more sustained release than the staeric acid and glycerylmonostearate due to its longer carbon chain length than the other two lipids. The lower melting point lipid can produce a controlled release from NLC. This is due to the presence of solid solution throughout the particle combined with the slow diffusion of drug from the lipid matrix (Priyanka and Sathali 2012).

The order of drug release from the three lipids as follows:

Stearic acid >Glycerylmonostearate>Compritol.

From the results it was concluded that higher lipid concentration decreases release rate. The nature of fatty acids affected the release significantly and longer the carbon chain length of fatty acids, the slower the drug release. From the results F27 formulation containing compritol ATO 888 using phospholipon 90G -3% was showed sustained release due to higher entrapment efficiency and long chain carbon atom of lipid.

Both burst release and sustained release are of interest for topical application. Burst release isuseful to improve the penetration of drug and for faster onset of action, while a sustainedrelease supplies the drug over a prolonged period of time.

5. Kinetics of drug release

The kinetics and mechanism of drug release were studied by release kinetics, the n, k and r^2 values are indicated in the Tables 6A – 6C. Among the models tested, the drug release profile of all formulations F1-F27 were best fitted with first order with r^2 values ranging from 0.854-0.993 and Higuchi model with r^2 values ranging from 0.987 - 0.994. From the results higuchi release kinetics showed purely diffusion controlled.

According to the data presented in the Tables 6A – 6C, the values of exponent n were in the range of 0.337- 0.551 which indicated that the drug release mechanism followed non-fickian model of release kinetics.

6. Particle size and zeta potential

Particle size distribution plays a critical role in influencing the physicochemical and penetration mechanism of drugs into the skin (Souto*et al.,* 2004). The particle size analysis of the NLC formulations was estimated by dynamic light scattering technique. Based on the entrapment efficiency and in vitrorelease profiles of NLC dispersions, Nine formulations, three formulation from each set of lipids (GMS &capryol – F3, F6, F9 and stearic acid & oleic acid – F12, F15, F18) and compritol& GMO - F21, F-24, F-27 at 3% , 5%, and 7% of lipid concentrations respectively) having higher entrapment and controlled release were selected for particle size analysis. Sizes of simvastatin loaded NLC were found to be in the range of 14.06 – 235.2 nm. The particle size values are shown in Table 7A-7C and the distribution curves were shown in Figure 6A- 6C.

i)Influence of lipid concentration on particle size:

The influence of different drug- lipid ratios of Simvastatin loaded nanostructured lipid carrier was investigated.

Formulations F3, F6 and F9 prepared using different concentration of lipid (glycerylmonostearate&caryol) (3%, 5%and 7%) containing Phospholipon 90G (3%) as stabilizer showed the particle size of 118 nm, 54.01 nm and 108.1nm respectively.

Formulations F12, F15 and F18 prepared using different concentration of lipid (staric acid & oleic acid) (3%, 5%and 7%) containing Phospholipon 90G (3%) as stabilizer showed the particle size of 53 nm, 14.06 nm and 44.05 nm respectively.

Formulations F21, F24 and F27 prepared using different concentration of lipid (compritol& GMO) (3%, 5%and 7%) containing Phospholipon 90G (3%) as stabilizer showed the particle size of 217.8 nm, 94.43 nm and 235.2 nm respectively.

Particle size significantly increased with increasing concentration. Huge augmentation of particle size was observed at 7% lipid concentration. It was observed that at a particular surfactant concentration (3%), an increase in the lipid content decreased the particle size at an optimum concentration (5%) after which the particle size was increased. This is because the sonication energy more efficiently distributed in the dilute dispersion (i.e., lower lipid concentration) than the concentrated dispersion (i.e., higher lipid dispersion), which produced smaller particles at lower lipid concentration (Surajit Das *et al*., 2012). Larger particle size with increase in lipid content could be attributed to viscosity of inner phase that affected the shearing capacity of homogenizer, decrease in emulsifying efficiency and increase in particle agglomeration.

From the results, it was further observed that the formulations containing 5% lipid concentration were selected and the particle sizes of other lipids at the same concentration were showed decrease particle size (glycerylmonostearate had the particle size of 54 nm where as stearic acid had 14.06 nm and compritol ATO 888 had 94.43 nm particle sizes.

Hence it was noted that the drug: lipid ratio was found to have a positive effect on particle size i.e. increase in lipid concentration resulted in larger mean particle sizes and broader size distributions.

ii) Effect of different lipids on the particle size of simvastatin NLC

There is a significant difference in the size of the particles with change in lipids. The decreasing order of particle size for the three lipids is as follows:

Compritol>Glycerylmonostearate> Stearic acid.

Of the three lipids used stearic acid containing formulations showed lesser mean particle size than the glycerylmonostearate and compritol. This may be due to the tendency of NLC to increase in size with the increase in carbon chain length of the lipids and higher melting point lipid (Compritol melting point, MP: 72°C, glycerylmonostearate MP: 60°C, and stearic acid MP: 55°C). Compritol has a higher melting point than GMS and Stearic acid, which results in slower lipid recrystallization from the hot homogenized condition resulting in increase in particle size. Hence, the higher the melting point of lipids the higher the particle size.

From the results, formulation containing stearic acid at 5 % concentration was optimized to get an NLC formulation in nanometric size range.

iii) Polydispersity index (PDI)

The PDI for all the formulations as shown in Table 7A-7C and Figure 6A-6C . The PDI were in the range of 0.138 – 0.402 which is smaller than 0.5, which indicates a relative homogenous dispersion.Polydispersity index indicate the width of the particle size distribution, which ranges from 0 to 1. Theoretically, monodisperse populations indicates PI = 0. However, PI < 0.2 was considered as narrow distribution and those greater than 0.5 indicate high homogenicity. (Surajit Das te al., 2012).

iv) Zeta potential (ZP)

The simvaststin loaded nanostructured lipid carrier were characterized to evaluate the effect of different lipids at different concentrations on surface charge of nanoparticles. The results were presented in Table 8 and Figure 7A – 7C.

Zeta potential of formulations F1-F27 prepared with three different set of lipids (GMS,-capryol, stearic acid-oleic acid, compritol-GMO) and phospholipon 90G (3%) as lipid phase surfactant showed negative zeta potential (-11.63 mV to -23.4 mV).

Zeta potential or electrophoretic mobility is an electric charge on particle surface forms electrical barrier which results in 'Repulsion phenomenon' is the zeta potential of a particular formulation (Shailesh S. Chalikwar., 2012). Most particles dispersed in an aqueous system will acquire a surface charge, principally either by ionization of surface groups, or adsorption of charged species. ZP is useful to predict the physical stability of NLC dispersion.

It has been reported, that in a combined electrostatic and sterical stabilization a zeta potential of about -20 mV can be sufficient for physical stability (Jacobs and Muller 2002).All nanoparticles showed a high negative residual charge due to chemical nature of the lipid matrix (stearic acid/oleic acid/behenic acid) and surfactant used. Hence NLC have potential of physical stability as it has zeta potential of -13.6 – 23.4 mV which is near to -20 mV. Values of f potential showed prepared NLC have sufficient charge and mobility to inhibit aggregation of particles.

7. Selection and evaluation of best formulation

Based on entrapment efficienc, *in vitro* release and particle size

Based on entrapment efficiency the formulations containing phospholipon 90G at 3% as surfactant showed higher entrapment on lipids. Due to high entrapment, drug release from NLCs was sustained. According to these release profile 9 formulations were selected. Among the 9 formulation 3 formulation showed smaller particle size at optimized 5% lipid concentration. From that F15 (stearic acid & oleic acid) was selected as the best formulation because of its smallest particle size.

a. Infrared (IR) spectroscopic studies

IR studies were carried out to confirm the compatibility between the drug, lipids and excipients for the above selected best formulations (F15). The IR peaks are shown in Figure 8. The major peak functional groups of simvastatin (3550.95, 3010.88, 2970.38, and 1724.36 cm-1) was obtained in the best formulations and no more additional of functional peaks were noticed.

b. Morphology of SLN by Scanning electron microscopy (SEM) technique:

The SEM photograph of the selected formulation F15 was shown in Figure 9. The SLN dispersion showed the particle size was found to be less than 1µm in size with the spherical shape and almost smooth surface.(Priyanka and Sathali 2012).

E. FORMULATION OF SIMVASTATIN LOADED NLC BASED HYDROGEL

The overall objective of the present work was to develop a hydrogel dosage form containing simvastatin as a potential dermatological formulation. The selected best NLC formulation (on the basis of highest entrapment efficiency, controlled *in vitro* release and smaller particle size among the all formulations) was incorporated into suitable gel base (Chitosan as gelling agent 4%) to obtain 0.5% of the drug. Plain simvastatin gel was prepared by incorporating the drug into gel base to obtain same 0.5% of the drug. Both the formulas were showed in Table 9.

F. EVALUATION OF THE PREPARED SIMVASTATIN NLC BASED HYDROGEL

1.Determination of Physicochemical properties

The physical properties of the gel formulations are shown in Table 10. The physical appearances of all gel formulations were yellow in colour, translucent,smooth and homogeneous in texture.

2. pH measurement

The results are shown in table No.10. The pH of the gel was measured in between 5.3 to 5.2 at intervals of 0,15 ,30 th day respectively which is compatible with normal skin pH in healthy people (Hadgraft J. *et al.,*2001)That may be rendered its topical application to skin without irritation. It is important to say that there was no significant change in the pH values as a function of time for all formulations.

3. Drug content

The drug content of the prepared gels was determined after lysing the nanoparticles with methanol. It was measured at 239 nm at UV-visible spectrophotometer, 1700-pharma spec, Japan. Drug content of the developed HG was found to be 96.51 % and 97.71% for NLC based gel and plain gel respectively. This indicated that the uniform distribution of drug in prepared gel formulations. The results were in shown Table 11.

4. Texture analysis

The mechanical parameters firmness and adhesiveness obtained by texture profile analysis for all tested semi-solid formulations are shown in Fig 10A – 10C.

An ideal semi-solid formulation for topicall delivery should have a combination of high adhesiveness, in order to prolong the contact time to allow for the drug release and appropriate firmness, to facilitatelocal application.These parameters are directly influenced by the formulation viscosity.

Gel Batch Code	Firmness (Kg)	Cohesiveness (Kg.sec)	Consistency (Kg)	Index of viscosity (Kg.sec)
PG	0.166±0.009	1.246±0.035	0.077± 0.006	0.404±0.05
NG	0.069±0.003	0.629±0.031	0.042±0.003	0.268±0.014
NLC (F15)	0.010±0.001	0.108±0.015	0.005±0.000	0.002±0.000

From the tableN.o. 12, data indicative that the viscosity of the optimized NLC based HG formulation was enough to adhere on skin surface which increase the residence time, enhances penetration across the skin and thereby increase drug availability at site of action in controlled manner and the subsequent systemic absorption.Even thoughthe plain HG is firmer, more cohesive and of a thicker consistency than NLC based HG.the presence of NLC within the HGs allowed for obtaining a topical application form having the desired semi-solid consistency, with major properties of firmness, adhesiveness and enhancedspreadability. (Serra L *et al.,* 2009,.Jones D.S *et al.,* 1997, Jones D.S *et al.,* 1999).

5. *Exvivo* Skin permeation study

Exvivo skinpermeation studies were performed to compare the permeation of drug from the optimized NLC, NLC Based HG and plain HG preparation from excised rat skin. The result of Skin permeation studies were portrayed in Figure 11and table No.13, which shown drug release in 12 hrs. All of the formulations contained the same amount of simvastatin (5mg).

Fig. 11 shows the drug release profiles of 31.01% and 27.44% for NLC and NLC based HG, respectively, in the first 2 hour whereas it was only 14.53% for plain HG. The cumulative % drug release at 12 hours was 64.54%, 61.08% and 46.66% for NLC, NLC based HG and plain HG respectively.

These differences could be explained by the higher viscosity of plain HG when compared to NLC based HG, since the drug release from semi-solid formulations is influenced by its rheological behaviour (Lippacher.A*et al.,* 2001).

Furthermore, The NLC dispersions and NLC based HG formulations possessed a sustained drug release over a period of 12 h, but the sustained effect was more pronounced with NLC based HG formulations compared to optimized NLC, which could be related with the presence of the gel matrix, simvastatin release from the NLC-based HGs should be further delayed, due to the combined diffusionalresistance of both systems. The slower release of drug from NLC and NLC based gels maintained the drug concentration for longer period of time (Bhaskar K *et al* 2009).

Moreover,The results of drug permeation from all the formulations through the rat abdominal skin confirmed that simvastatin was released and permeated through the rat skin and hence could possibly permeate through the human skin.

6. Drug deposition study

The result of skin deposition study was indicated in table n.o. 14. The Percentage of drug remained on the skin from NLC dispersion, NLC based HG and plain gel was found to be 15.01%, 12.56% and 41.11% respectively, which shows that plain drug HG had poor penetration through the skin.

Percentage of drug retained into the skin from NLC dispersion, NLC based HG and plain gel was found to be 17.62%, 23.44% and 9.13% respectively. This shows the better penetration ability of NLC based HG and it was more than that of plain gel and NLC dispersions. Thus, drug-localizing effect in the skin seems possible with novel colloidal particulate drug carriers such as NLC. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin. So from above result, it has been concluded that NLCBG induce better result had better compared to others (Pallavi V *et al.,* 2006).

7. *Ex vivo* release kinetics

To analyze the drug release mechanism in vitro release data was fitted into various release equations and kinetic models (Zero order, First order, Hixson – Crowell, Higuchi and Korsemeyer – Peppas models). The release kinetic data for all the formulations were shown in theTable15 and Figure 12A to 28E.

Among the models tested, the drug release profile of all formulations F1-F27 were best fitted with first order with r^2 values ranging from 0.992-0.995 and Higuchi model with r^2 values ranging from 0.986 - 0.995. From Table 15it can be concluded that, all formulations fitted very well to the Korsmeye–Peppas model with different R^2 and n values, which means that the drug release processes were different. Classical Fickian diffusion was the release mechanism for NLC dispersion with n value of 0.432 and anomalous non-Fickian (combination of diffusion and erosion mechanisms) for both NLC based HG and plain HG with n value of 0.49 and 0.7 respectively. (Ritger P.L. *et al.,* 1987).

8. Invivo Diabetic Wound healing activity

Results of In-vivo wound healing studies were represented in figure 14A & 14B. From the figure, it could be noticed that all NLC based HG treated groups showed a time dependant increase in % wound contractions which is significantly higher than any of the treated groups .

By day 4, NLC based HG treated wounds were 48.29% healed compared with 41.34% and 26.80% in the plain gel treated and control group respectively.

Eighty percent wound closure could be achieved within 7 days for those groups treated with NLC based HG. In contrast, control wounds with no drug healed more slowly and about 80% wound closure was achieved only after 12 days (Fig. 14A & 14B). There was no significant different between NLC based HG and plain gel treated groups (96.42% wound closure by day 12). It was also revealed that none of the rats in simvastatin treated groups showed scab formation but rats in control group showed formation of scab which delayed wound healing.

The findings indicate that the wound healing in both NLC based HG and plain gel treated groups occurred faster than the control group, while complete wound closure, hair began to grow and the wounds area were completely covered with hair was observed on 12^{th} day of wound induction in NLC based HG treated groups revealed that the NLC based HG had the superiority over other tested groups.

The results of the present study indicate that simvastatin promote the healing in diabetic wounds. This fast and higher wound contraction rate may be ascribed to the dual effect of the drug and the vehicle, chitosan HG.

The prohealing effects of these drugs may be due to their proliferative activity, enhanced angiogenesis & endothelial nitric oxide release leading to increased blood flow in growing tissue. Its antioxidant property may also contribute to itsprohealing effect. Chitosan induced the migration of the inflammatory cells, formation of granulation tissue or re-epithelialization in the early stages of wound healing. And whenglycerin applied to the skin, signals the cells to mature in normal fashion, a hydrogel dressing of high glycerin content is better and more suitable to manage wound healing. In addition, glycerin is known to have humectants (Xiangjian Z *et al.,* 2003, Okan G *et al.,* 2011)

9. Skin irritation study

The results of the skin irritation study revealed that the Nanostructured lipid carrier based gel exhibited considerably no signs of irritation or erythemas were noticed and all the animals tolerated the applied gels during the whole period of study. Therefore, it can be assured that the gel formulation can be used for topical application.

TABLES AND FIGURES

TABLE 1

CALIBRATION OF SIMVASTATIN IN pH 7.4 SALINE PHOSPHATE BUFFER CONTAINING 0.15% SDS

S. NO	CONCENTRATION (µg/ml)	ABSORBANCE ± SD*
1	2	0.118 ±0.0151
2	4	0.241 ±0.0127
3	6	0.351 ±0.0104
4	8	0.460 ±0.0172
5	10	0.613 ±0.0115
6	12	0.716 ±0.0116
7	14	0.842 ±0.0145
8	16	0.964 ±0.0030
9	18	1.066 ±0.0053
10	20	1.182 ±0.019

n=3*

γ = 0.99961

TABLE 2A: COMPOSITION OF SIMVASTATIN NLC

FORMULATION CODE	LIPID COMPOSITION		SOLID LIPID : LIQUID LIPID RATIO	DRUG -LIPID RAIO	SURFACTANT CONCENTRATION	
	SOLID LIPID	LIQUID LIPID			PHOSPOLIPON (% W/V)	TWEEN-80 (% W/V)
F1	GMS	CAPRYOL	70:30	1:3	1%	2%
F2	GMS	CAPRYOL	70:30	1:3	2%	2%
F3	GMS	CAPRYOL	70:30	1:3	3%	2%
F4	GMS	CAPRYOL	70:30	1:5	1%	2%
F5	GMS	CAPRYOL	70:30	1:5	2%	2%
F6	GMS	CAPRYOL	70:30	1:5	3%	2%
F7	GMS	CAPRYOL	70:30	1:7	1%	2%
F8	GMS	CAPRYOL	70:30	1:7	2%	2%
F9	GMS	CAPRYOL	70:30	1:7	3%	2%

TABLE 2B: COMPOSITION OF SIMVASTATIN NLC

FORMULATION CODE	LIPID COMPOSITION		SOLID LIPID : LIQUID LIPID RATIO	DRUG -LIPID RAIO	SURFACTANT CONCENTRATION	
	SOLID LIPID	LIQUID LIPID			PHOSPOLIPON (% W/V)	TWEEN-80 (% W/V)
F10	STEARIC ACID	OLEIC ACID	70:30	1:3	1%	2%
F11	STEARIC ACID	OLEIC ACID	70:30	1:3	2%	2%
F12	STEARIC ACID	OLEIC ACID	70:30	1:3	3%	2%
F13	STEARIC ACID	OLEIC ACID	70:30	1:5	1%	2%
F14	STEARIC ACID	OLEIC ACID	70:30	1:5	2%	2%
F15	STEARIC ACID	OLEIC ACID	70:30	1:5	3%	2%
F16	STEARIC ACID	OLEIC ACID	70:30	1:7	1%	2%
F17	STEARIC ACID	OLEIC ACID	70:30	1:7	2%	2%
F18	STEARIC ACID	OLEIC ACID	70:30	1:7	3%	2%

TABLE 2C: COMPOSITION OF SIMVASTATIN NLC

FORMULATION CODE	LIPID COMPOSITION		SOLID LIPID : LIQUID LIPID RATIO	DRUG -LIPID RAIO	SURFACTANT CONCENTRATION	
	SOLID LIPID	LIQUID LIPID			PHOSPOLIPON (% W/V)	TWEEN-80 (% W/V)
F19	COMPRITOL	GMO	70:30	1:3	1%	2%
F20	COMPRITOL	GMO	70:30	1:3	2%	2%
F21	COMPRITOL	GMO	70:30	1:3	3%	2%
F22	COMPRITOL	GMO	70:30	1:5	1%	2%
F23	COMPRITOL	GMO	70:30	1:5	2%	2%
F24	COMPRITOL	GMO	70:30	1:5	3%	2%
F25	COMPRITOL	GMO	70:30	1:7	1%	2%
F26	COMPRITOL	GMO	70:30	1:7	2%	2%
F27	COMPRITOL	GMO	70:30	1:7	3%	2%

TABLE 3

DRUG CONTENT OF SIMVASTATIN NLC

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD
1	F1	94.07±0.771
2	F2	94.39±1.082
3	F3	94.50±0.412
4	F4	94.01±1.083
5	F5	93.52±0.577
6	F6	94.13±0.429
7	F7	93.25±0.730
8	F8	94.72±0.249
9	F9	94.01±1.083
10	F10	94.74±0.871
11	F11	93.37±0.737
12	F12	93.47±0.911
13	F13	92.87±0.408
14	F14	93.36±0.820
15	F15	94.23±0.836
16	F16	93.63±1.020
17	F17	93.47±0.863
18	F18	93.31±0.433
19	F19	94.29±0.714
20	F20	93.58±0.836
21	F21	93.96±0.587
22	F22	93.69±0.677
23	F23	93.15±0.592
24	F24	94.02±0.736
25	F25	94.50±0.578
26	F26	93.96±0.432
27	F27	93.25±0.408

n=3*

TABLE 4A: ENTRAPMENT EFFICIENCY OF SIMVASTATIN NLC CONTAINING GLYCERYL MONO STEARATE & CAPRYOL

FORMULATION CODE	LIPID (%)	SURFACTANT % W/V	ENTRAPMENT EFFICIENCY (%) ± SD
F1	3	1	29.38±2.260
F2	3	2	37.62±2.041
F3	3	3	41.26±1.255
F4	5	1	33.43±0.616
F5	5	2	40.07±2.263
F6	5	3	43.14±0.917
F7	7	1	40.74±2.504
F8	7	2	49.37±0.571
F9	7	3	54.13±0.867

n=3*

TABLE 4B: ENTRAPMENT EFFICIENCY OF SIMVASTATIN NLC CONTAINING STEARIC ACID & OLEIC ACID

FORMULATION CODE	LIPID (%)	SURFACTANT % W/V	ENTRAPMENT EFFICIENCY (%) ± SD
F10	3	1	25.61±1.642
F11	3	2	34.54±1.117
F12	3	3	41.87±1.377
F13	5	1	29.18±1.007
F14	5	2	37.38±0.629
F15	5	3	40.64±1.358
F16	7	1	37.77±1.076
F17	7	2	43.52±1.453
F18	7	3	49.72±0.449

n=3*

TABLE 4C: ENTRAPMENT EFFICIENCY OF SIMVASTATIN NLC CONTAINING COMPRITOL & GLYCERYL MONO OLEATE

FORMULATION CODE	LIPID (%)	SURFACTANT % W/V	ENTRAPMENT EFFICIENCY (%) ± SD
F19	3	1	41.98±1.228
F20	3	2	46.70±2.306
F21	3	3	52.84±1.423
F22	5	1	52.12±1.011
F23	5	2	58.44±1.332
F24	5	3	62.16±1.606
F25	7	1	56.05±2.142
F26	7	2	63.24±2.435
F27	7	3	67.76±1.523

n=3*

TABLE 5A: COMPARISON OF INVITRO RELEASE PROFILE OF SIMVASTATIN LOADED NLC CONTAINING GLYCERYL MONO STEARATE & CAPRYOL LIPIDS

TIME (HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD								
	F1 LIPID 3% + 1% Phospolipon	F2 LIPID 3% + 2% Phospolipon	F3 LIPID 3% + 3% Phospolipon	F4 LIPID 5% + 1% Phospolipon	F5 LIPID 5% + 2% Phospolipon	F6 LIPID 5% + 3% Phospolipon	F7 LIPID 7% + 1% Phospolipon	F8 LIPID 7% + 2% Phospolipon	F9 LIPID 7% + 3% Phospolipon
0.5	23.07±0.77	16.99±1.56	15.53±0.60	18.66±0.76	14.92±1.69	13.58±0.84	14.58±2.05	12.23±0.69	10.67±0.89
1	29.27±1.28	23.01±1.63	21.29±0.86	24.38±0.71	21.05±2.12	19.47±0.68	20.93±2.29	18.28±0.78	16.03±1.28
1.5	34.76±1.54	28.55±2.39	25.91±0.69	29.49±0.87	25.84±2.49	24.36±0.77	25.89±2.64	23.43±0.69	21.11±1.39
2	39.63±1.58	32.80±3.63	30.58±0.63	34.69±0.91	30.89±2.92	29.18±1.02	30.73±2.37	28.36±0.56	26.01±1.40
3	42.82±1.84	36.42±2.99	34.40±0.92	38.50±1.01	34.50±2.79	33.09±0.71	34.38±3.12	31.88±0.70	29.73±1.07
4	45.97±1.99	40.52±3.09	37.65±0.67	42.34±1.12	37.74±2.05	36.66±0.62	38.74±2.82	35.55±0.71	33.59±0.90
5	49.49±2.14	44.49±3.69	41.58±1.01	45.93±0.97	41.18±1.91	40.04±0.77	42.30±2.85	38.74±0.96	37.45±0.43
6	53.09±1.74	47.72±3.06	45.51±1.19	49.67±0.67	44.82±2.41	43.38±0.82	45.67±3.13	42.02±1.14	40.71±0.19
7	56.17±1.67	51.58±3.06	49.35±1.08	53.33±0.57	48.32±2.36	46.87±0.99	49.07±2.64	45.49±1.00	43.89±0.42
8	59.22±0.61	56.15±2.24	53.11±1.19	56.97±0.74	51.63±2.65	50.22±1.18	53.11±3.27	48.95±1.01	47.28±0.49
9	63.86±0.46	59.87±1.77	57.31±1.23	60.86±0.86	55.25±2.49	53.77±1.10	57.07±3.31	52.59±0.86	50.29±0.85
10	67.41±0.50	64.07±1.83	60.75±0.69	64.61±0.87	59.29±2.37	57.46±0.78	60.69±3.43	56.11±0.76	53.62±1.01
11	71.45±0.31	67.79±1.44	64.34±0.70	68.18±1.06	63.53±2.39	61.07±0.76	64.94±3.08	59.37±1.18	56.64±1.31
12	75.81±1.43	71.05±1.96	67.84±0,61	71.99±0.96	67.75±2.52	64.72±0.86	68.62±3.15	62.72±1.36	59.79±1.09

n=3*

TABLE 5B: COMPARISON OF INVITRO RELEASE PROFILE OF SIMVASTATIN LOADED NLC CONTAINING STEARIC ACID & OLEIC ACID LIPIDS

TIME (HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD								
	F10 LIPID 3% + 1% Phospolipon	F11 LIPID 3% + 2% Phospolipon	F12 LIPID 3% + 3% Phospolipon	F13 LIPID 5% + 1% Phospolipon	F14 LIPID 5% + 2% Phospolipon	F15 LIPID 5% + 3% Phospolipon	F16 LIPID 7% + 1% Phospolipon	F17 LIPID 7% + 2% Phospolipon	F18 LIPID 7% + 3% Phospolipon
0.5	26.71(±)2.88	20.28(±)3.56	15.42(±)2.11	22.13(±)2.09	17.94±0.42	15.70(±)2.43	16.62(±)0.59	12.85(±)2.61	10.33(±)2.37
1.0	32.79(±)3.36	26.41(±)3.24	21.44(±)1.87	27.99(±)2.20	23.93±0.51	21.78(±)2.63	22.66(±)0.59	18.85(±)2.39	16.14(±)2.16
1.5	37.92(±)3.25	31.59(±)3.49	26.57(±)1.67	33.13(±)2.24	29.31±0.59	27.14(±)2.58	27.74(±)0.84	24.15(±)2.54	21.41(±)2.29
2.0	43.16(±)3.23	36.76(±)3.53	31.59(±)1.71	38.09(±)2.19	34.57±0.81	32.21(±)2.67	32.63(±)0.97	29.08(±)2.56	26.32(±)2.32
3.0	46.82(±)3.29	40.53(±)3.49	35.25(±)1.69	41.77(±)2.19	38.49±1.00	36.11(±)2.79	36.64(±)1.21	32.88(±)2.12	30.09(±)1.87
4.0	50.52(±)3.41	44.45(±)3.73	39.34(±)1.88	45.69(±)2.03	42.10±0.92	39.75(±)3.07	40.35(±)1.46	36.73(±)2.13	33.91(±)1.88
5.0	54.19(±)2.96	48.06(±)3.21	43.13(±)2.13	49.77(±)2.05	45.97±0.72	43.55(±)2.96	44.26(±)1.48	40.19(±)2.27	37.34(±)2.02
6.0	58.12(±)3.04	51.38(±)3.02	46.78(±)1.94	53.88(±)2.10	49.94±0.72	47.54(±)2.97	48.20(±)0.78	44.18(±)2.18	41.30(±)1.92
7.0	62.02(±)3.01	55.11(±)2.75	50.92(±)2.08	57.75(±)2.45	53.93±0.59	51.41(±)3.16	52.35(±)0.79	47.95(±)2.43	45.05(±)2.18
8.0	65.52(±)3.12	58.87(±)2.48	54.98(±)2.13	61.54(±)2.40	57.97±0.76	55.36(±)3.09	56.21(±)0.56	51.85(±)2.58	48.51(±)1.72
9.0	69.21(±)3.26	62.39(±)2.36	58.79(±)1.93	65.58(±)2.35	61.87±0.82	59.41(±)3.13	60.26(±)1.04	55.86(±)2.59	52.91(±)2.33
10.0	73.04(±)2.85	65.99(±)2.27	62.54(±)1.95	69.55(±)2.22	65.75±0.66	63.26(±)3.40	64.43(±)1.64	59.57(±)2.62	56.59(±)2.35
11.0	76.90(±)2.63	69.39(±)2.37	66.58(±)1.96	73.44(±)2.34	69.60±0.75	67.09(±)3.45	68.38(±)1.18	63.31(±)2.17	60.31(±)1.90
12.0	80.35(±)2.24	72.72(±)2.12	69.83(±)1.95	77.25(±)2.45	73.43±0.59	70.85(±)2.89	72.28(±)1.31	66.75(±)1.95	63.64(±)1.56

n=3*

TABLE 5C: COMPARISON OF INVITRO RELEASE PROFILE OF SIMVASTATIN LOADED NLC CONTAINING COMPRITOL & GLYCERYL MONO OLEATE LIPIDS

TIME (HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD								
	F19 LIPID 3% + 1% Phospolipon	**F20 LIPID 3% + 2% Phospolipon**	**F21 LIPID 3%+3% Phospolipon**	**F22 LIPID 5% + 1% Phospolipon**	**F23 LIPID 5% + 2% Phospolipon**	**F24 LIPID 5% + 3% Phospolipon**	**F25 LIPID 7% + 1% Phospolipon**	**F26 LIPID 7% + 2% Phospolipon**	**F27 LIPID 7% + 3% Phospolipon**
0.5	13.63±1.93	12.29±0.79	11.68±2.87	12.96±1.01	10.39±0.76	9.61±1.24	11.12±3.06	9.44±0.84	8.27±0.35
1.0	19.63±1.87	17.50±0.77	16.66±3.13	18.23±1.03	15.91±0.85	14.90±1.41	16.37±3.29	14.68±0.85	13.38±0.35
1.5	25.09±1.97	22.48±0.86	21.29±3.08	22.94±1.04	20.66±0.85	19.19±1.74	21.11±3.39	19.13±1.12	17.59±0.58
2.0	30.03±1.99	27.29±1.36	25.69±3.03	27.69±0.79	25.28±0.70	23.46±2.14	25.85±3.42	23.45±1.37	21.68±0.43
3.0	33.79±1.87	31.13±1.38	29.36±3.08	31.04±0.88	28.65±0.87	26.42±1.95	29.18±3.20	26.31±1.78	24.46±0.69
4.0	37.69±1.96	34.68±1.24	33.11±3.13	34.36±0.72	32.01±0.81	29.76±2.37	32.43±3.28	29.64±1.94	27.39±0.53
5.0	41.25±1.81	38.48±1.24	36.56±2.85	37.93±0.63	35.28±0.77	33.12±2.23	35.98±3.56	33.00±1.94	30.27±0.72
6.0	45.05±1.85	42.26±1.43	40.04±2.78	41.65±0.73	38.64±0.66	36.90±2.15	39.57±3.24	36.45±1.77	33.64±0.81
7.0	48.84±2.09	45.79±1.37	43.39±2.67	45.07±0.79	41.97±0.65	40.38±1.89	42.97±3.03	39.92±1.63	37.21±1.06
8.0	52.88±2.02	49.80±1.29	46.87±2.28	48.46±0.73	45.28±0.66	43.45±2.49	46.34±2.47	43.33±1.75	40.69±0.84
9.0	56.67±1.86	53.29±1.2	50.50±2.29	52.10±0.70	48.61±0.86	46.38±2.37	49.96±2.37	46.25±1.89	43.93±0.83
10.0	60.51±1.97	56.98±1.05	53.77±2.19	55.45±0.92	51.86±0.96	49.78±2.17	53.45±1.87	49.54±1.63	47.19±0.86
11.0	64.17±1.86	60.76±1.06	57.46±1.80	58.76±1.25	55.25±0.88	53.09±2.17	56.91±1.67	53.08±1.73	50.32±0.86
12.0	67.82±1.86	64.06±1.06	61.29±1.74	62.49±1.09	58.56±0.86	56.61±2.09	60.63±1.85	56.59±1.64	53.81±0.91

n=3*

TABLE 6A: RELEASE KINETICS OF SIMVASTATIN LOADED NLC CONTAINING GLYCERYL MONOSTEARATE & CAPRYOL

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F1	0.876	4.707	0.980	-0.038	0.987	17.56	0.988	0.353	0.985	-0.104
F2	0.924	4.823	0.990	-0.036	0.992	18.73	0.993	0.433	0.992	-0.104
F3	0.931	4.657	0.993	-0.033	0.993	18.19	0.994	0.448	0.993	-0.097
F4	0.910	4.776	0.990	-0.036	0.994	18.38	0.994	0.411	0.991	-0.103
F5	0.926	4.557	0.986	-0.032	0.989	17.77	0.992	0.448	0.987	-0.094
F6	0.935	4.570	0.989	-0.031	0.992	18.25	0.988	0.523	0.986	-0.093
F7	0.931	4.709	0.990	-0.034	0.992	18.47	0.994	0.462	0.990	-0.099
F8	0.929	4.367	0.990	-0.029	0.993	17.25	0.992	0.484	0.986	-0.081
F9	0.935	4.274	0.991	-0.027	0.996	17.08	0.994	0.518	0.987	-0.084

TABLE 6B: RELEASE KINETICS OF SIMVASTATIN LOADED NLC CONTAINING STEARIC ACID & OLEIC ACID LIPIDS

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F10	0.865	4.994	0.987	-0.045	0.993	18.48	0.991	0.337	0.991	-0.118
F11	0.900	4.939	0.988	-0.040	0.993	18.86	0.994	0.394	0.991	-0.110
F12	0.930	4.823	0.992	-0.036	0.993	18.90	0.994	0.458	0.992	-0.102
F13	0.901	5.026	0.988	-0.042	0.993	19.10	0.992	0.383	0.992	-0.114
F14	0.919	4.942	0.990	-0.038	0.993	19.15	0.993	0.428	0.991	-0.108
F15	0.928	4.858	0.990	-0.036	0.993	19.01	0.994	0.454	0.991	-0.104
F16	0.929	4.926	0.989	-0.038	0.992	19.21	0.993	0.445	0.992	-0.107
F17	0.939	4.702	0.992	-0.033	0.993	18.63	0.994	0.494	0.991	-0.097
F18	0.950	4.598	0.992	-0.031	0.993	18.45	0.992	0.541	0.991	-0.093

TABLE 6C: RELEASE KINETICS OF SIMVASTATIN LOADED NLC CONTAINING COMPRITOL & GLYCERYL MONO OLEATE LIPIDS

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F19	0.935	4.730	0.991	-0.034	0.993	18.67	0.994	0.481	0.991	-0.099
F20	0.944	4.542	0.993	-0.031	0.994	18.05	0.995	0.501	0.992	-0.092
F21	0.944	4.311	0.992	-0.028	0.993	17.13	0.995	0.501	0.991	-0.085
F22	0.935	4.333	0.992	-0.029	0.993	17.08	0.994	0.474	0.991	-0.086
F23	0.940	4.146	0.991	-0.026	0.993	16.51	0.992	0.513	0.993	-0.097
F24	0.949	4.044	0.993	-0.025	0.993	16.17	0.993	0.527	0.991	-0.077
F25	0.944	4.276	0.991	-0.028	0.992	17.01	0.993	0.507	0.990	-0.084
F26	0.950	4.040	0.992	-0.023	0.992	16.17	0.992	0.531	0.990	-0.077
F27	0.956	3.882	0.854	-0.056	0.990	15.60	0.990	0.551	0.990	-0.073

TABLE 7A: PARTICLE SIZE OF SIMVASTATIN NLC CONTAINING GMS-CAPRYOL

FORMULATION CODE	LIPID (%)	MEAN DIAMETER (nm)	PDI
F3	3%	118	0.257
F6	5%	54.01	0.138
F9	7%	108.1	0.313

TABLE 7B: PARTICLE SIZE OF SIMVASTATIN NLC CONTAINING STEARIC ACID- OLEIC ACID

FORMULATION CODE	LIPID (%)	MEAN DIAMETER (nm)	PDI
F12	3%	53	0.295
F15	5%	14.06	0.347
F18	7%	44.05	0.402

TABLE 7C: PARTICLE SIZE OF SIMVASTATIN NLC CONTAINING COMPRITOL-GMO

FORMULATION CODE	LIPID (%)	MEAN DIAMETER (nm)	PDI
F21	3%	217.8	0.401
F24	5%	94.43	0.274
F27	7%	235.2	0.258

TABLE 8: ZETA POTENTIAL OF SIMVASTATIN NLC CONTAINING PHOSPHOLIPON 90G - 3% AS SURFACTAN

S. NO	FORMULATION CODE	ZETA POTENTIAL (mV)
1	F3	-18.2
2	F6	-21.7
3	F9	-14.2
4	F12	-11.63
5	F15	-13.6
6	F18	-16.2
7	F21	-23.4
8	F24	-19.2
9	F27	-15.6

TABLE 9: COMPOSITION OF SIMVASTATIN HYDROGEL

SL. NO	INGREDIENTS	PLAIN GEL	NLC Based HG
1.	Chitosan	2g	2g
2.	Acetic Acid	1%	1%
3.	Glycerine	1.5 ml	1.5 ml
4.	Water	50 ml	50 ml
5.	Drug	300 mg	25 ml

TABLE 10: APPEARANCE AND pH OF FORMULATIONS

FORMULATIONS	APPEARANCE	pH at 0 day	pH at 15 th day	pH at 30 th day
NLC Based HG	Yellow	5.3	5.2	5.2
Plain HG	Yellow	5.2	5.2	5.2

n=3*

TABLE 11: DRUG CONTENT OF HYDROGEL FORMULATION

S.NO	FORMULATION	% DRUG CONTENT
1	NLC Based HG	96.51±0.74
2	Plain HG	97.71±0.43

n=3*

TABLE 12: TEXTURE ANALYSIS OF GEL FORMULATIONS

Gel Batch Code	Firmness (Kg)	Cohesiveness (Kg.sec)	Consistency (Kg)	Index of viscosity (Kg.sec)
Plain HG	0.166±0.009	1.246±0.035	0.077± 0.006	0.404±0.050
NLC Based HG	0.069±0.003	0.629±0.031	0.042±0.003	0.268±0.014
NLC (F15)	0.010±0.001	0.108±0.015	0.005±0.000	0.002±0.000

n=3*

TABLE 13: *EXVIVO* SKIN PERMEATION STUDY

TIME IN HOURS	CUMULATIVE PERCENTAGE OF DRUG RELEASED		
	NLC (F15)	NLC BASED HG (NG)	PLAIN HG (PG)
0.5	15.58±1.58	11.79±1.11	4.63±0.83
1	21.23±1.09	17.54±1.05	7.82±0.77
1.5	26.21±1.14	22.74±1.02	11.01±0.58
2	31.01±1.07	27.44±1.02	14.53±1.17
3	34.31±1.16	31.18±0.67	17.21±1.93
4	38.28±1.40	34.54±0.84	19.67±1.09
5	41.52±1.46	37.83±0.67	23.69±1.42
6	44.82±1.14	41.41±0.68	27.05±1.42
7	48.45±1.36	44.98±0.69	30.01±1.02
8	52.03±1.26	48.34±0.76	33.19±0.68
9	55.27±1.17	51.69±0.79	36.83±0.84
10	58.57±0.98	54.99±0.76	39.96±0.76
11	61.81±1.30	58.23±0.42	43.14±0.63
12	64.54±1.07	61.08±0.25	46.66±1.02

n=3*

TABLE 14: DRUG DEPOSITION STUDY

FORMULATION	% DRUG REMAINED ON SKIN AFTER 12 HOUR	% DRUG RETAINED INTO SKIN AFTER 12 HOUR
NLC (F15)	15.01±0.75	17.62±0.75
NLC BASED HG (NG)	12.56±0.59	23.44±1.48
PLAIN HG (PG)	41.11±1.34	9.13±0.49

n=3*

TABLE 15: COMPARISION OF *EX-VIVO* RELEASE KINETICS PROFILES

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		HIXSON-CROWELL		KORSMEYER-PEPPAS	
	R^2	$K_O^{(h-1)}$	R^2	$K_1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	$KHC^{(h-1/3)}$	R^2	n value
NLC (F 15)	0.971	3.911	0.993	-0.030	0.995	17.03	0.989	-0.089	0.995	0.432
NLC Based HG	0.970	3.914	0.992	-0.028	0.994	17.05	0.987	-0.085	0.993	0.491
Plain HG	0.993	3.465	0.995	-0.020	0.986	14.85	0.996	-0.066	0.995	0.700

TABLE 16: COMPARISION OF PERCENTAGE WOUND CONTRACTION OF DIFFERENT GROUPS

S.No	Day of Observation	Wound Contraction (%)		
		Control	Plain HG	NLC Based HG
2	4	26.80±0.60%	41.34±1.13%	48.29±0.69%
3	7	48.54±1.59%	73.49±1.16%	81.94±1.16%
4	10	62.74±1.37%	84.71±0.62%	94.96±0.35%
5	12	78.69±1.60%	96.42±1.39%	100%***

n=6*

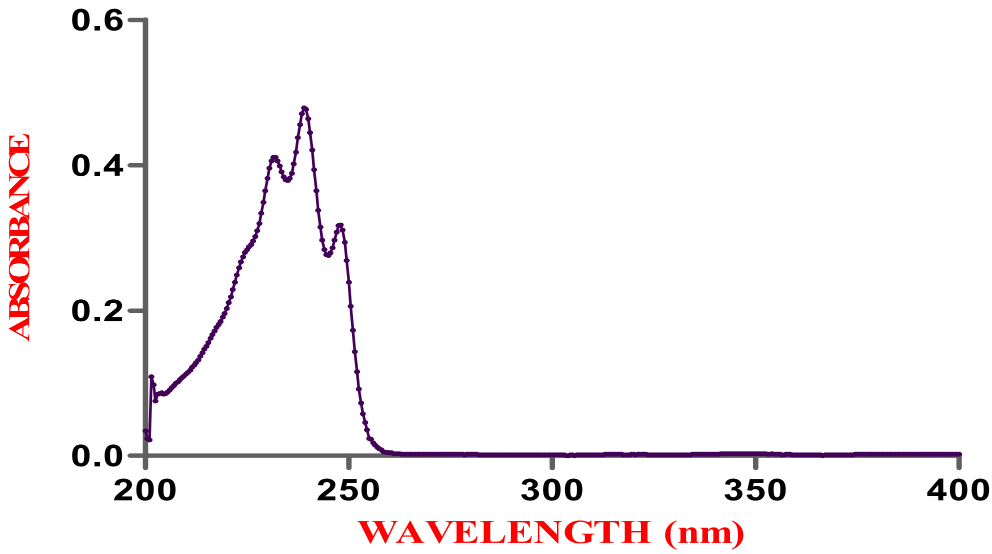


FIGURE 1A: DETERMINATION OF λ_{max} OF SIMVASTATIN IN SALINE PHOSPHATE BUFFER pH 7.4 CONTAINING 0.15% SDS

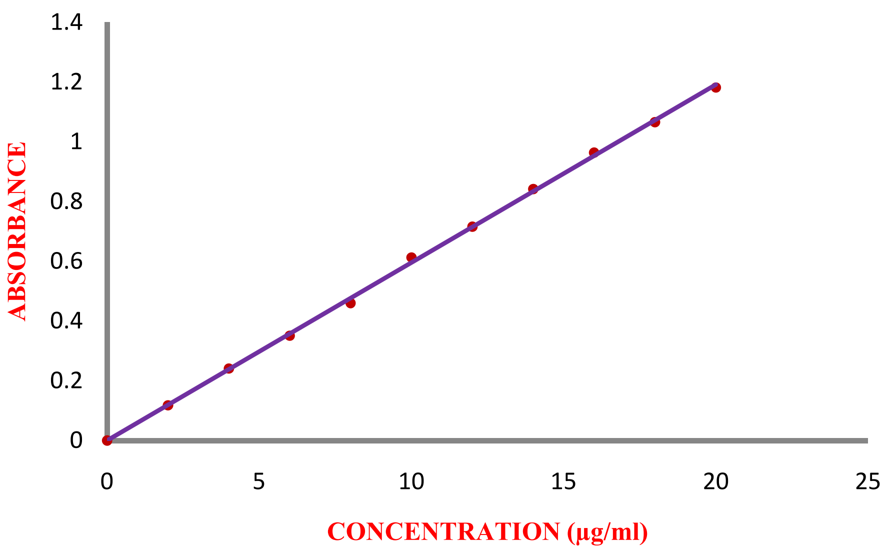


FIGURE 1B : CALIBRATION CURVE OF SIMVASTATIN USING PHOSPHATE BUFFER PH 7.4 CONTAINING 0.15% SDS

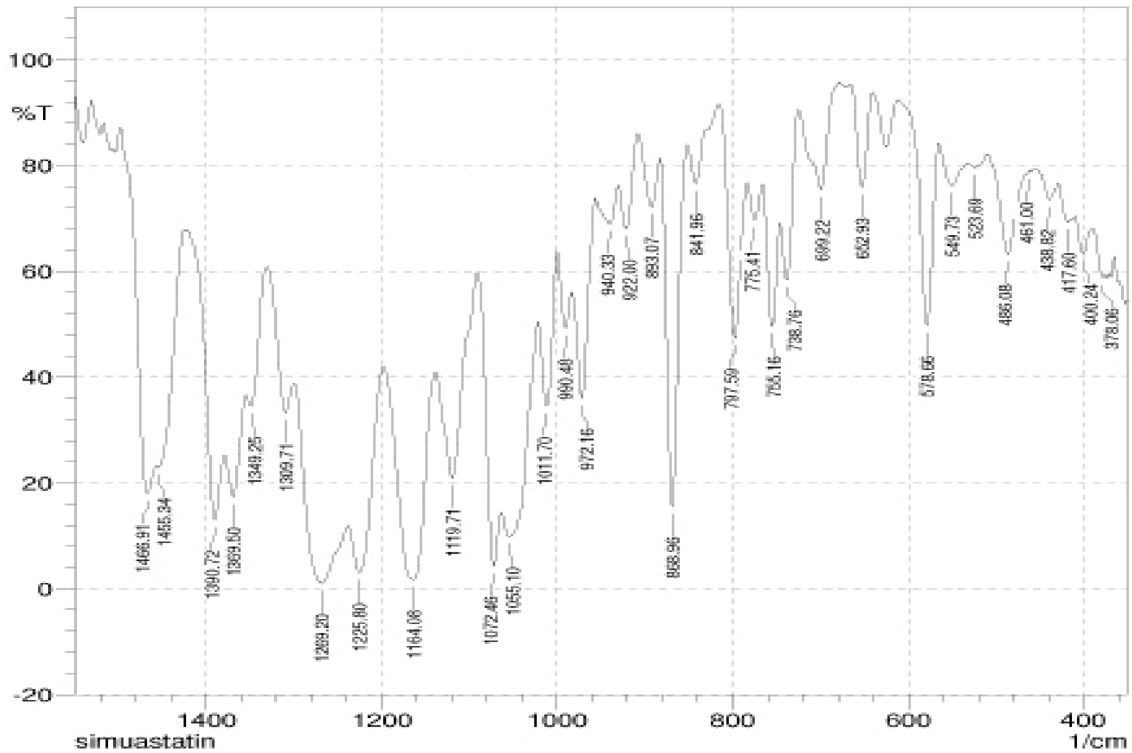


FIGURE 2A: FT-IR SPECTRA OF SIMVASTATIN

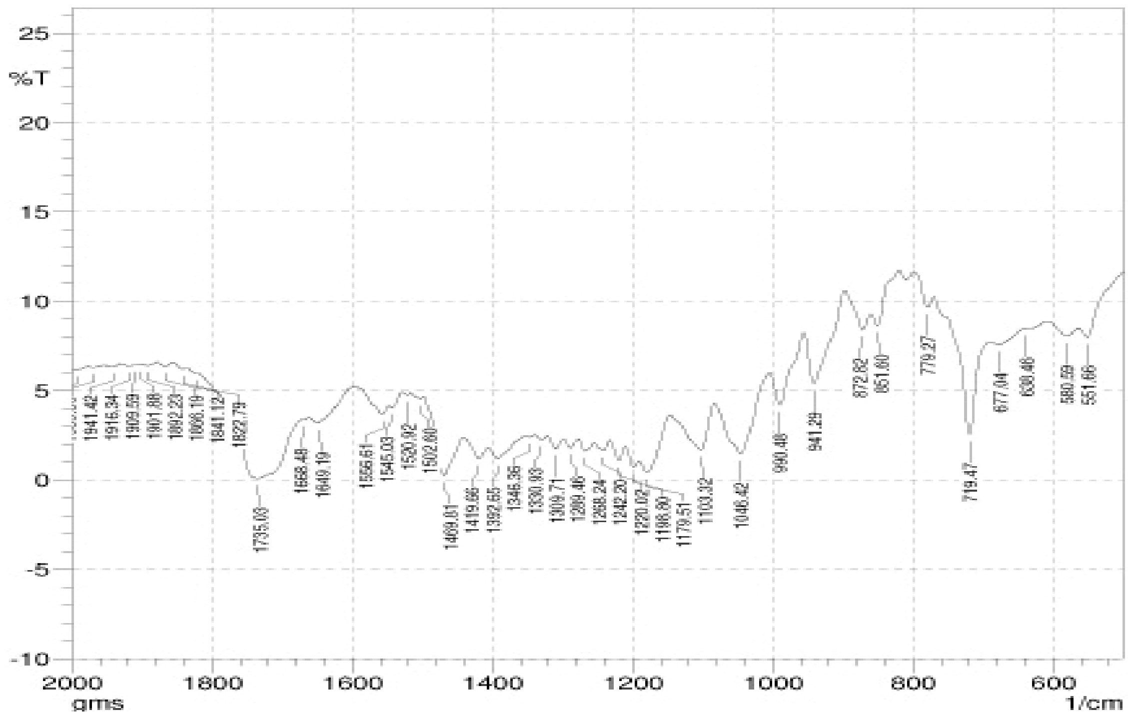


FIGURE 2B: FT-IR SPECTRA OF GLYCERYL MONO STEARATE

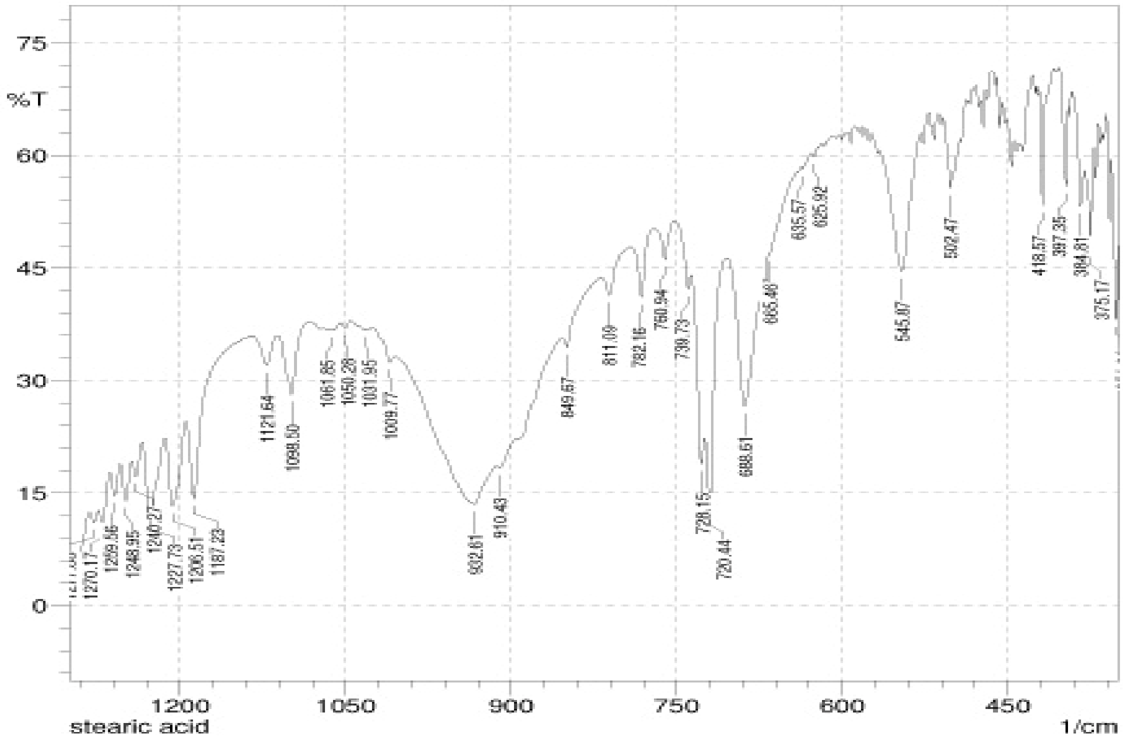


FIGURE 2C: FT-IR SPECTRA OF SREARIC ACID

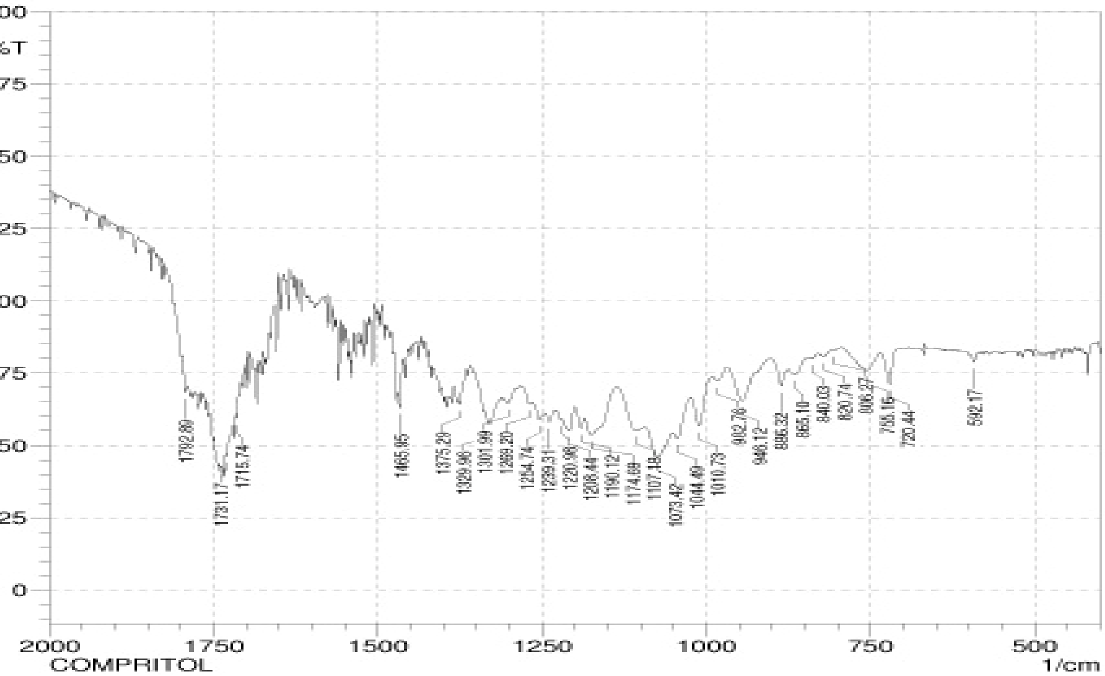


FIGURE 2D: FT-IR SPECTRA OF GLYCERYL BEHENATE

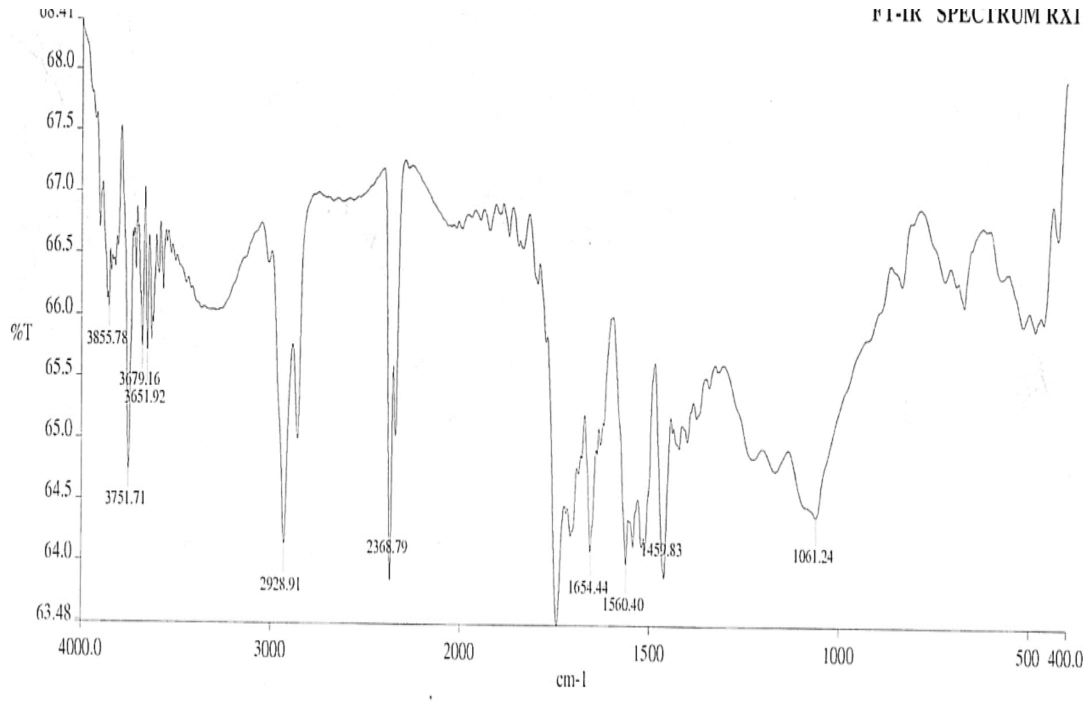


FIGURE 2E: FT-IR SPECTRA OF PHOSPHOLIPON 90G

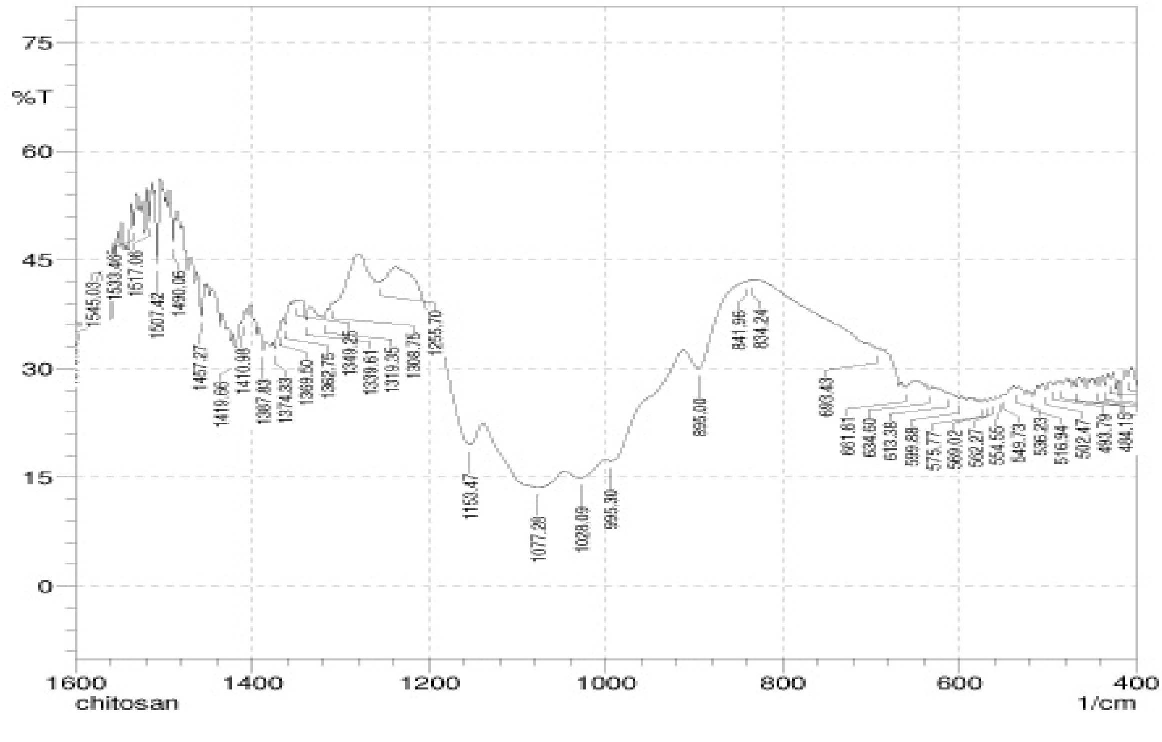


FIGURE 2F FT-IR SPECTRA OF CHITOSAN

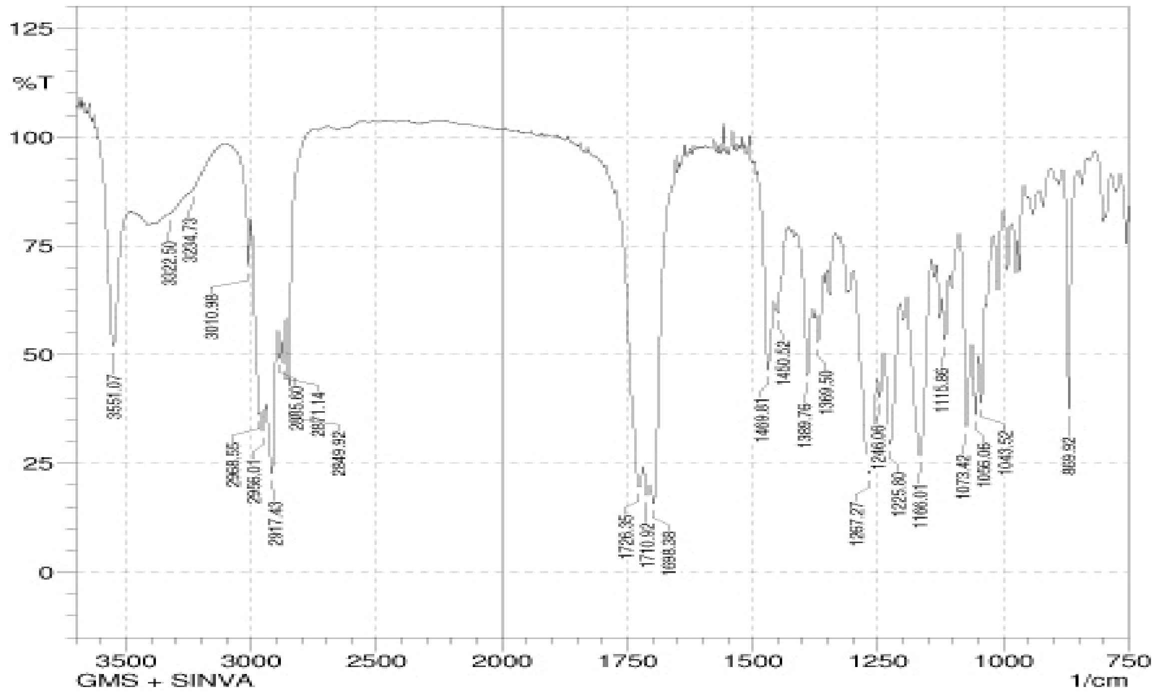


FIGURE 2G: FT-IR SPECTRA OF PHYSICAL MIXURE OF SIMVASTATIN + GLYCERYL MONO STEARATE + CHITOSAN

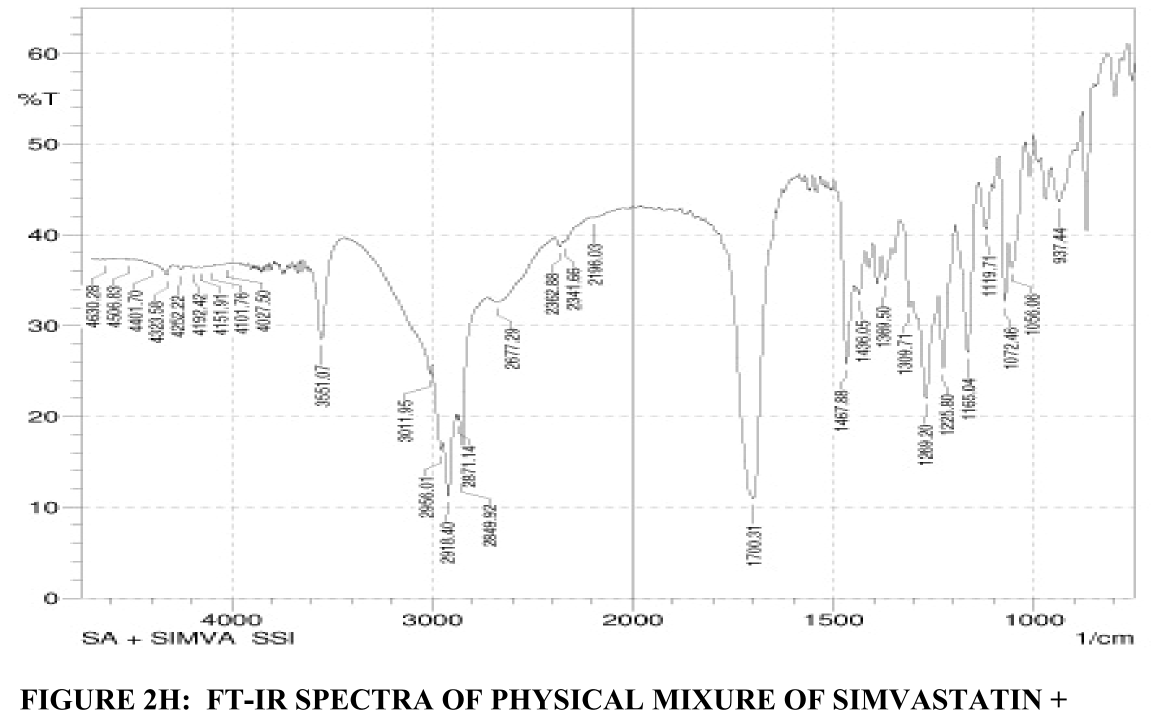


FIGURE 2H: FT-IR SPECTRA OF PHYSICAL MIXURE OF SIMVASTATIN + STEARIC ACID + CHITOSAN

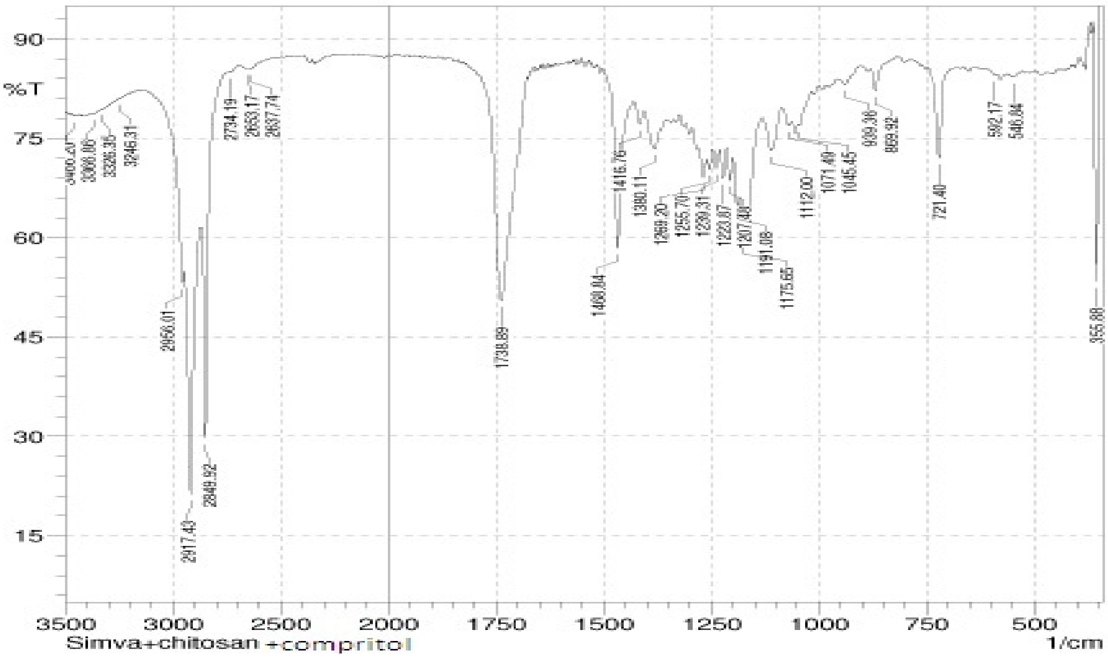


FIGURE 2I: FT-IR SPECTRA OF PHYSICAL MIXURE OF SIMVASTATIN + COMPRITOL + CHITOSAN

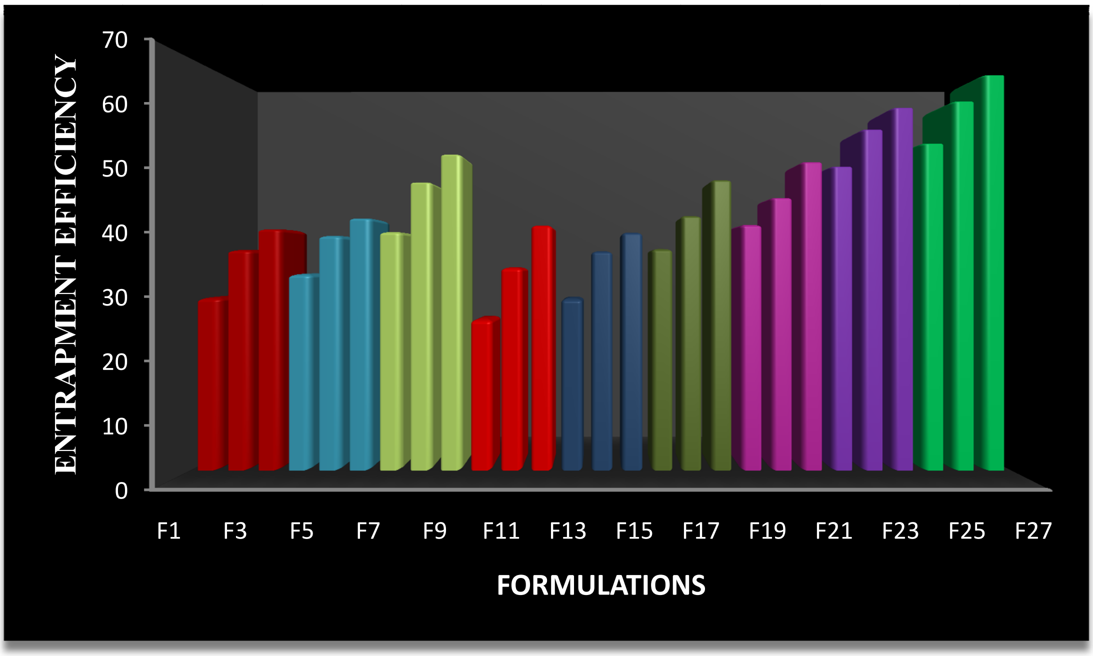


FIGURE 3: EFFECT OF DIFFERENT LIPIDS AT DIFFERENT CONCENTRATIONS ON THE DRUG ENTRAPMENT EFFICIENCIES OF SIMVASTATIN LOADED SOLID LIPID NANOPARTICLES

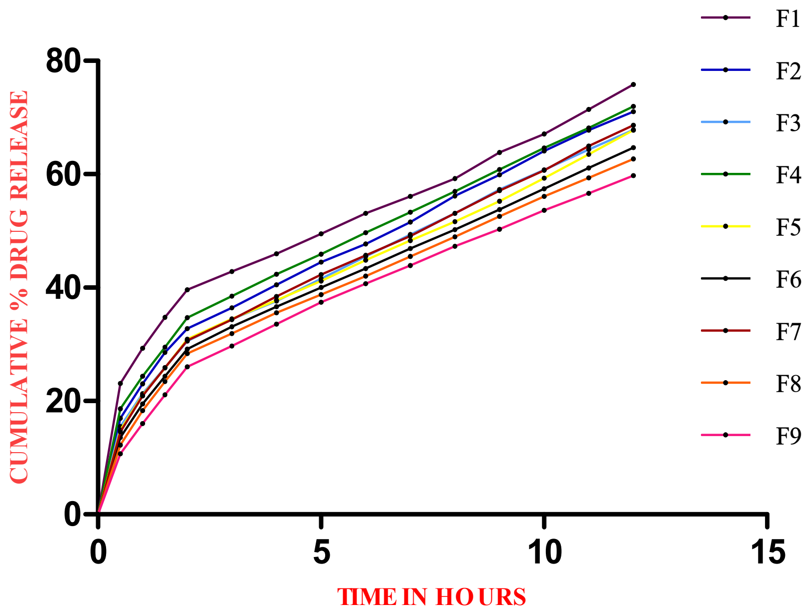


FIGURE 4A COMPARISON OF *INVITRO* RELEASE PROFILE OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL LIPIDS

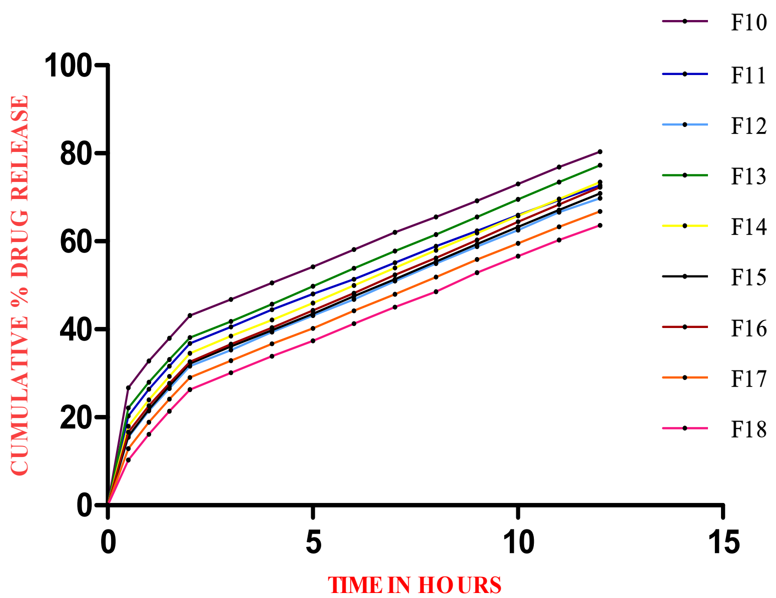


FIGURE 4B: COMPARISON OF *INVITRO* RELEASE PROFILE OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIES CONTAINING STEARIC ACID AND OLEIC ACID LIPIDS

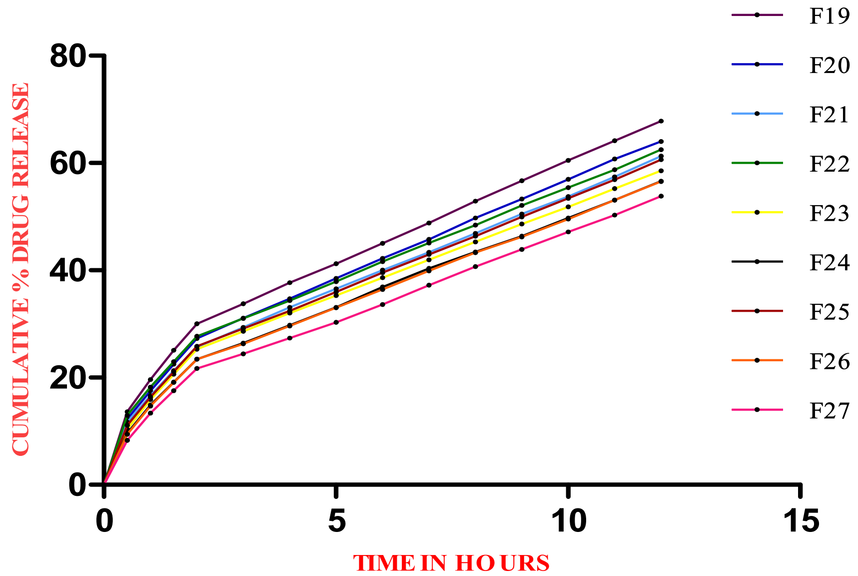


FIGURE 4C: COMPARISON OF *INVITRO* RELEASE PROFILE OF SIMVASTATIN LOADED NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS

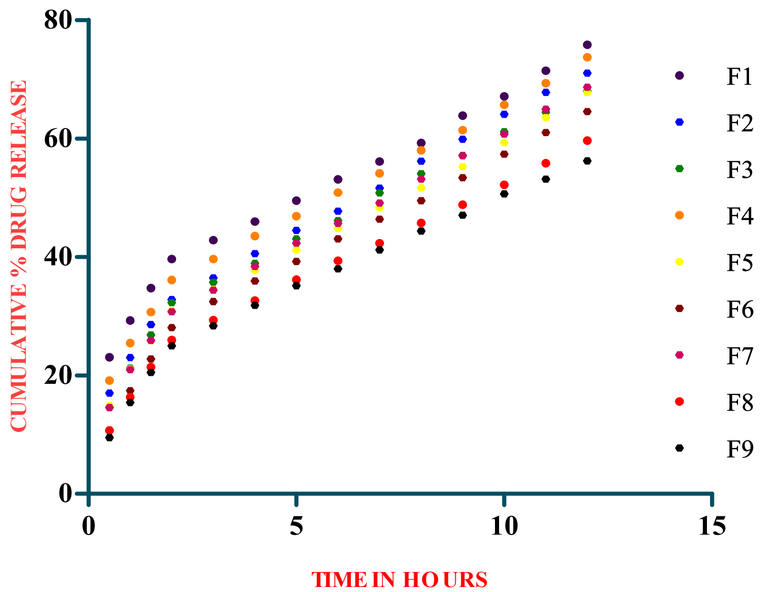


FIGURE 5A COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL LIPIDS

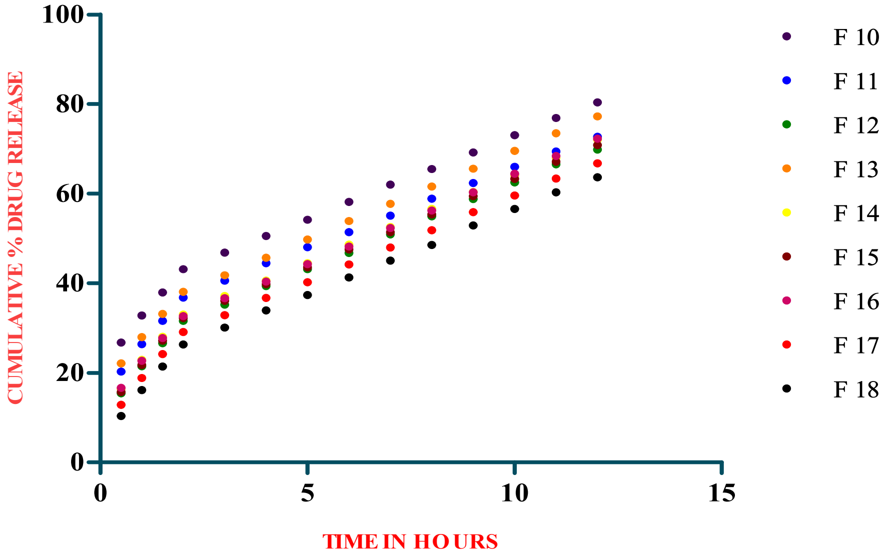


FIGURE 5B: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING STEARIC AID AND OLEIC ACID LIPIDS

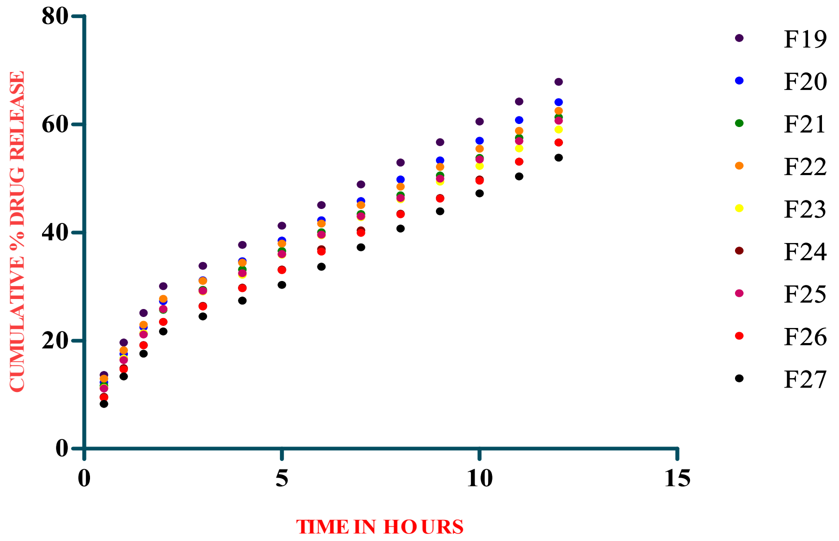


FIGURE 5C: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS

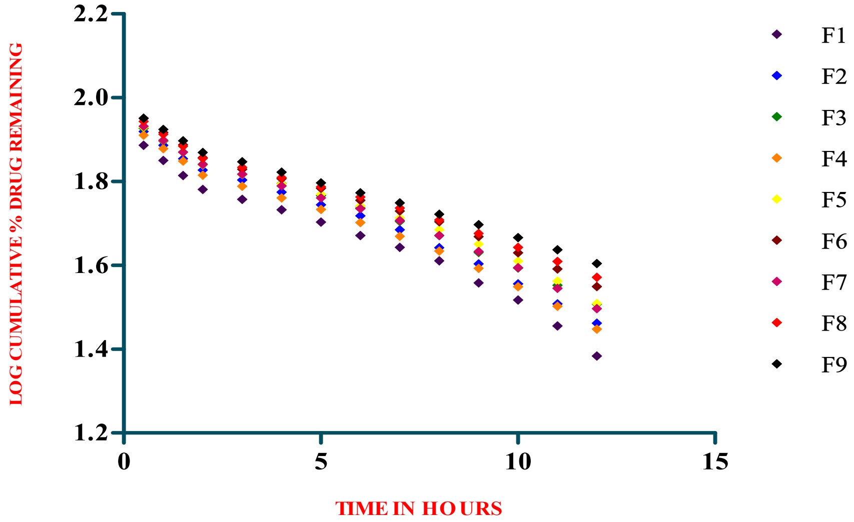


FIGURE 5D: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL LIPIDS

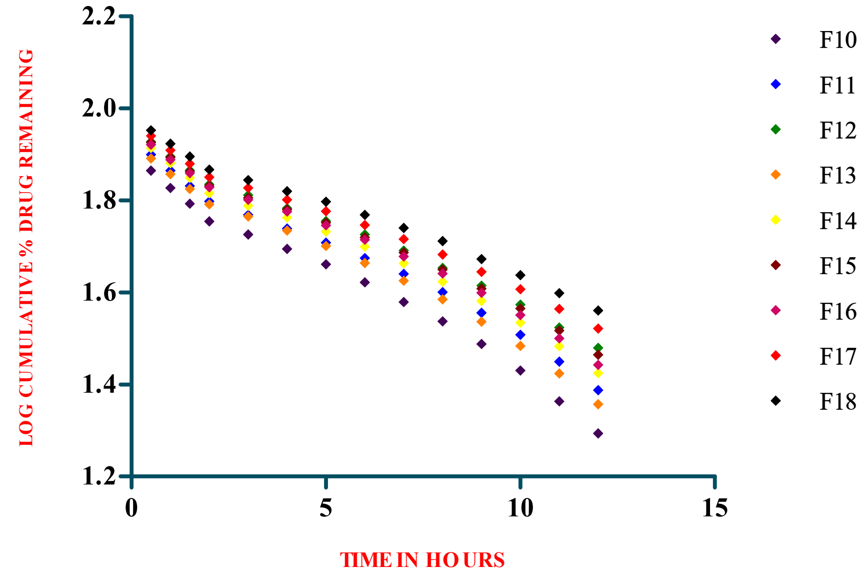


FIGURE 5E: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING STEARIC ACID AND OLEIC ACID LIPIDS

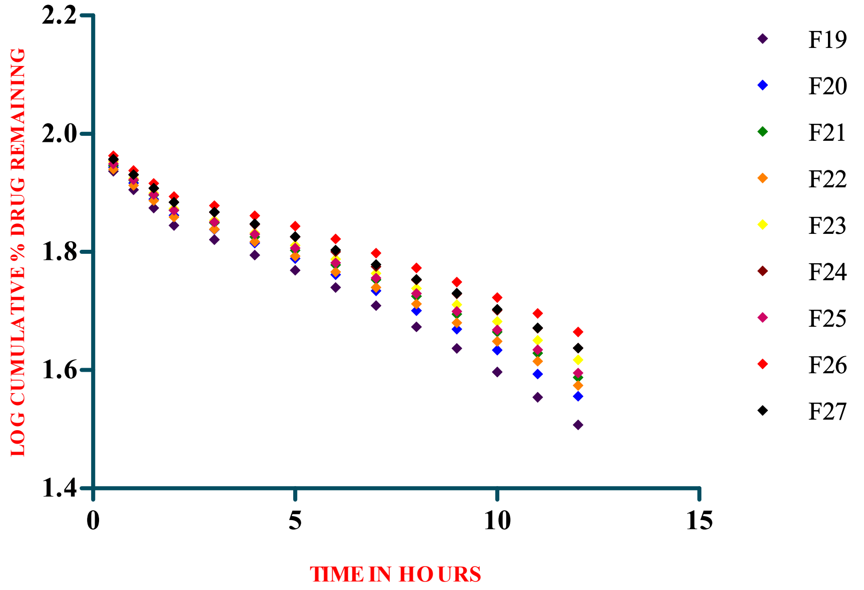


FIGURE 5F: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS

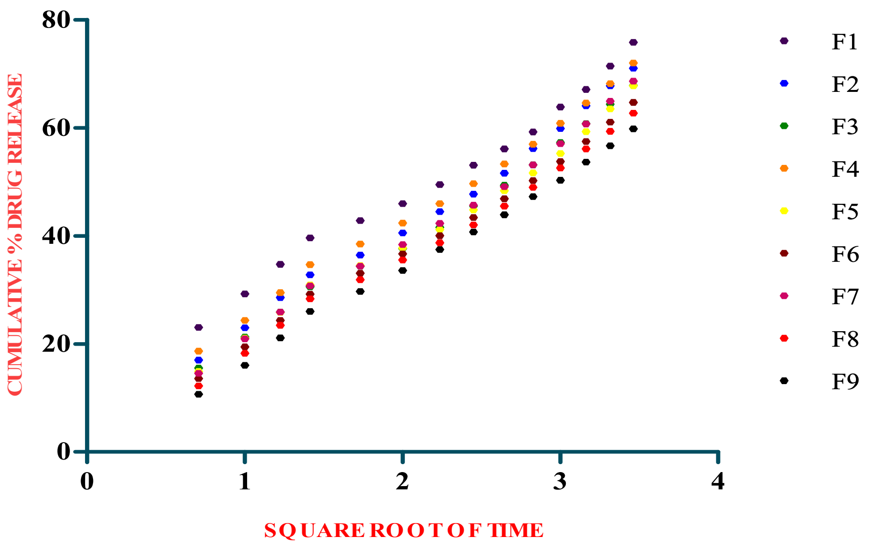


FIGURE 5G: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL

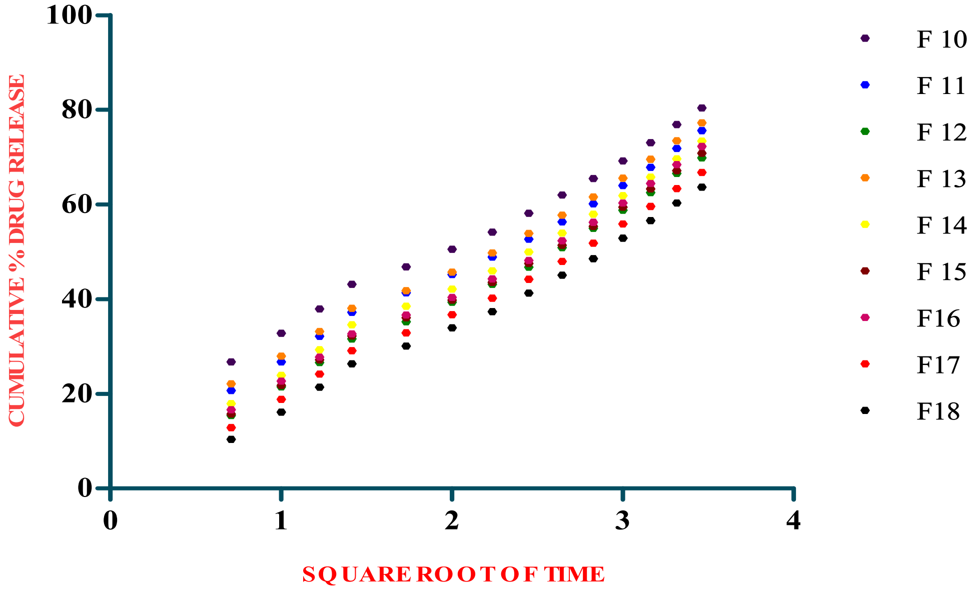


FIGURE 5H: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING STEARIC ACID AND OLEIC ACID LIPIDS

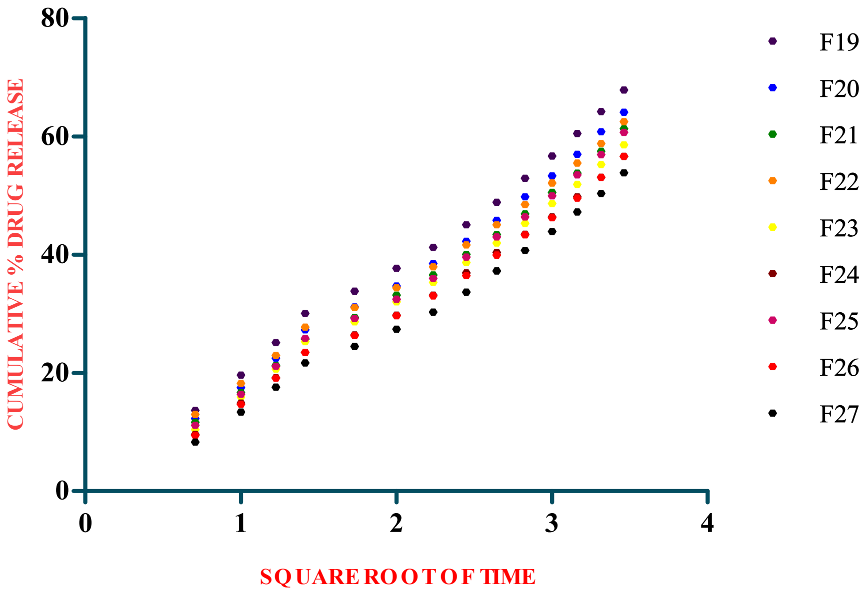


FIGURE 5I: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS

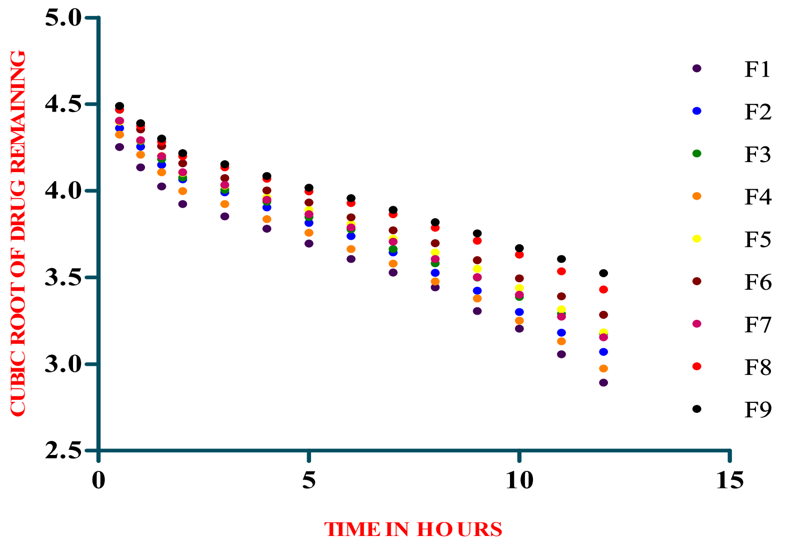


FIGURE 5J: COMPARISON OF *INVITRO* HIXSON-CROWELL MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL

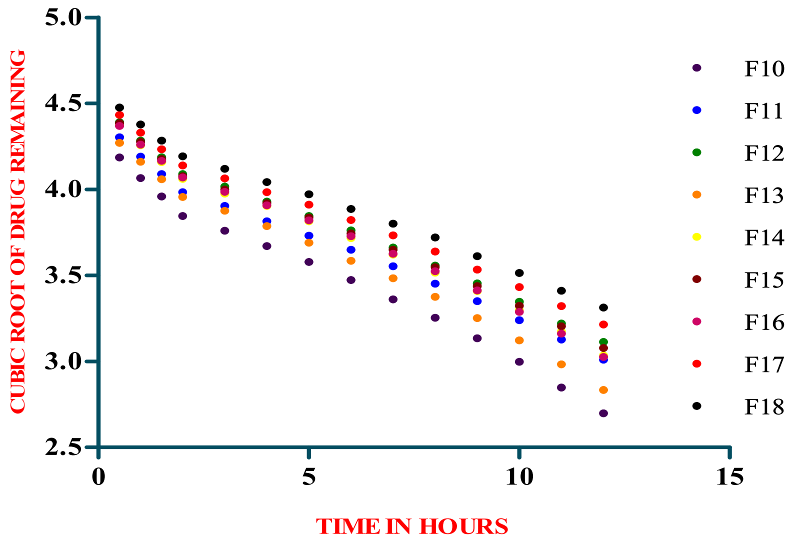


FIGURE 5K: COMPARISON OF *INVITRO* HIXSON-CROWELL MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING STEARIC ACID AND OLEIC ACID LIPIDS

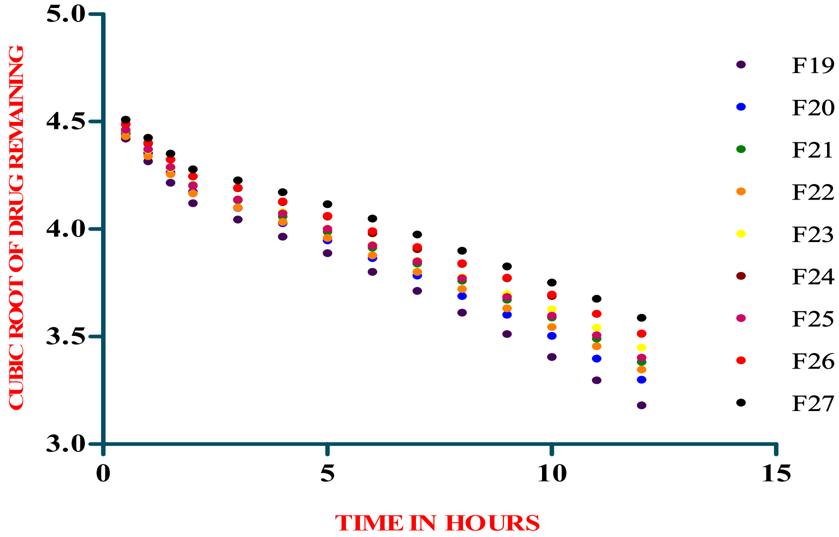


FIGURE 5L: COMPARISON OF *INVITRO* HIXSON-CROWELL MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS

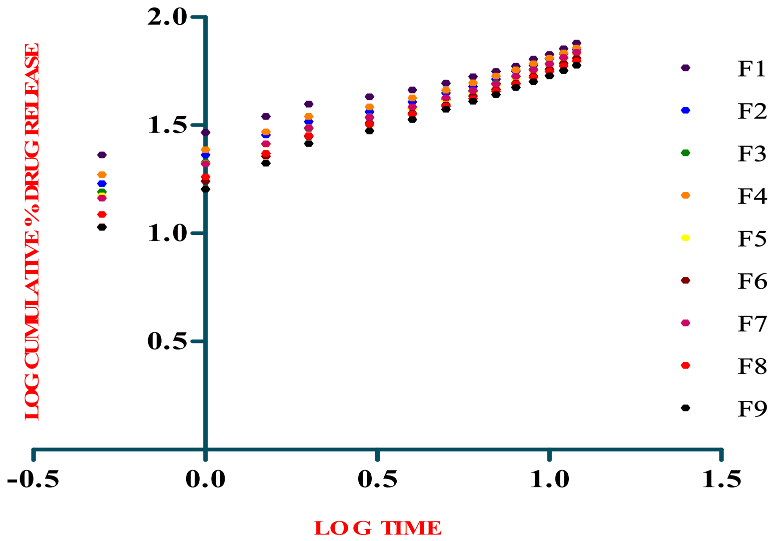


FIGURE 5M: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL MONOSTEARATE AND CAPRYOL

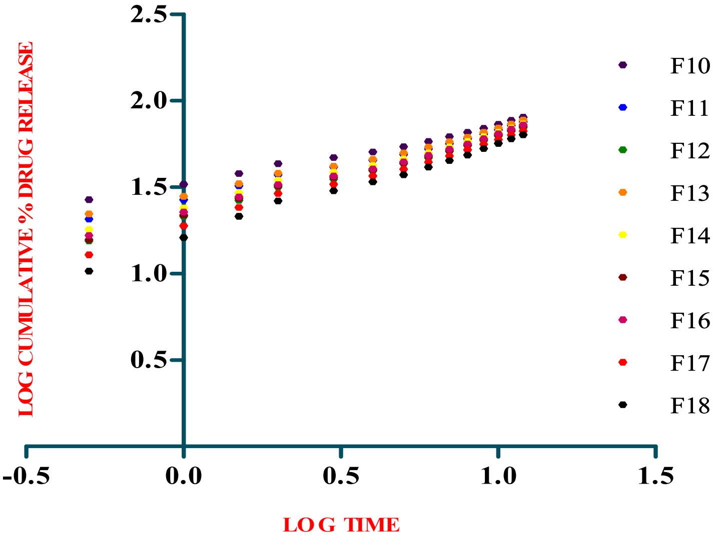


FIGURE 5N: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF NANOSTRUCTURED LIPID CARRIERS CONTAINING STEARIC ACID AND OLEIC ACID LIPIDS

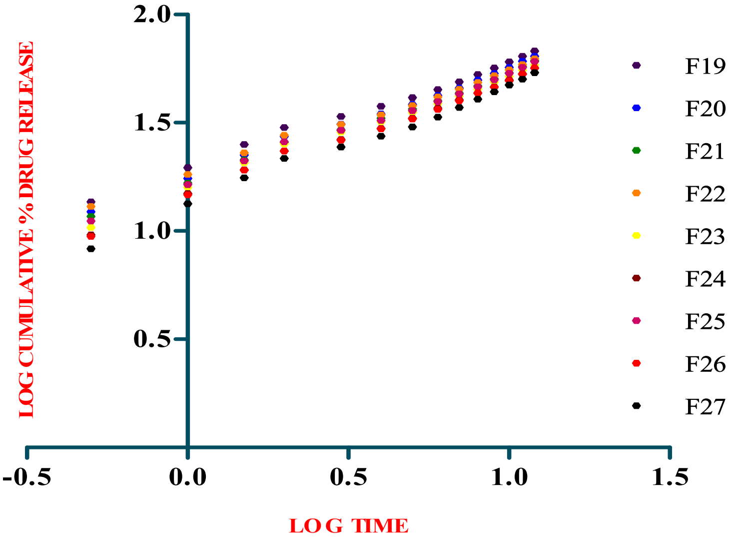
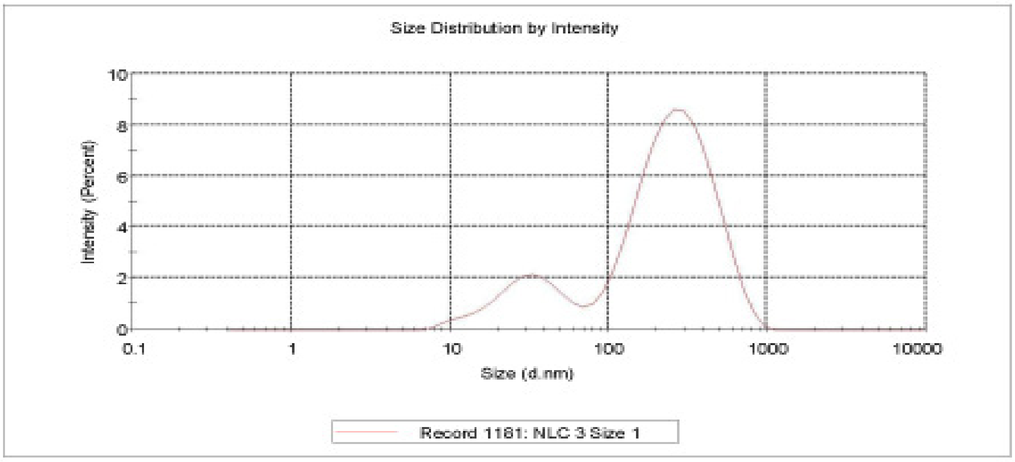
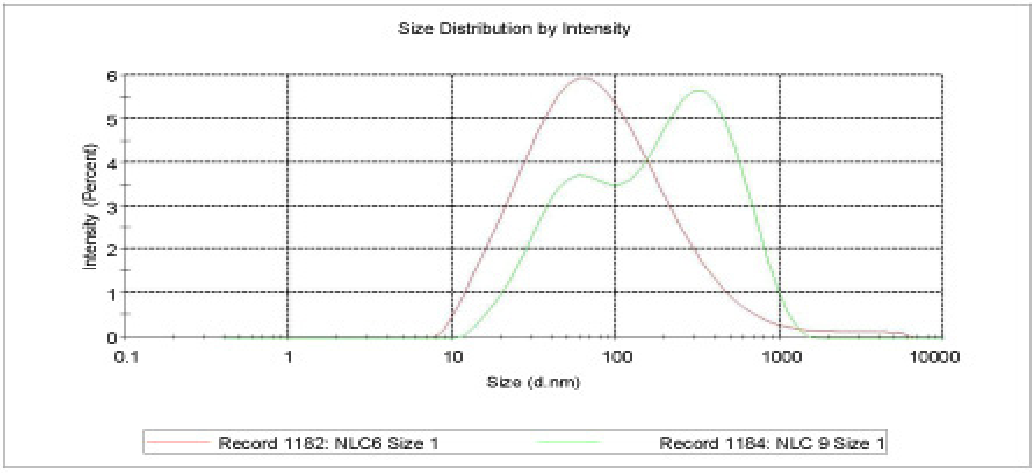


FIGURE 5O: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE NANOSTRUCTURED LIPID CARRIES CONTAINING GLYCERYL BEHENATE AND GLYCERYL MONO OLEATE LIPIDS





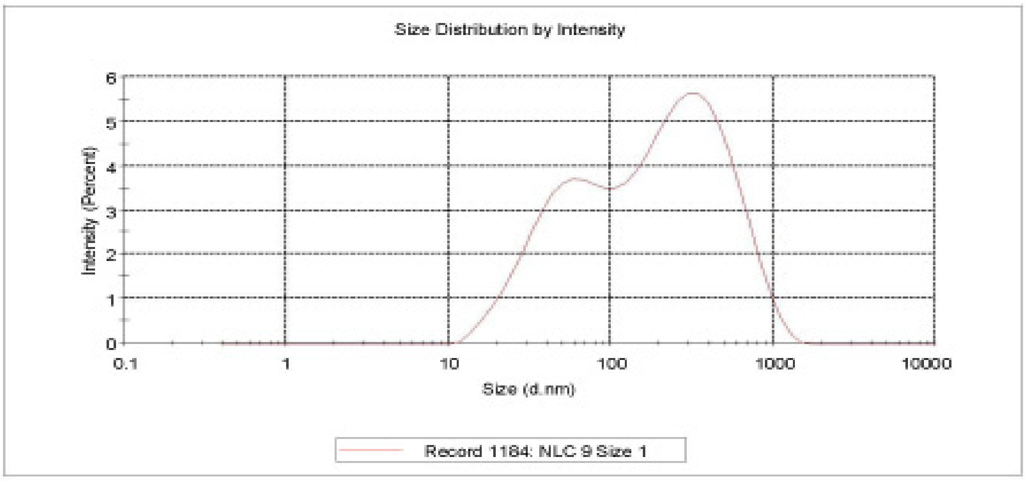
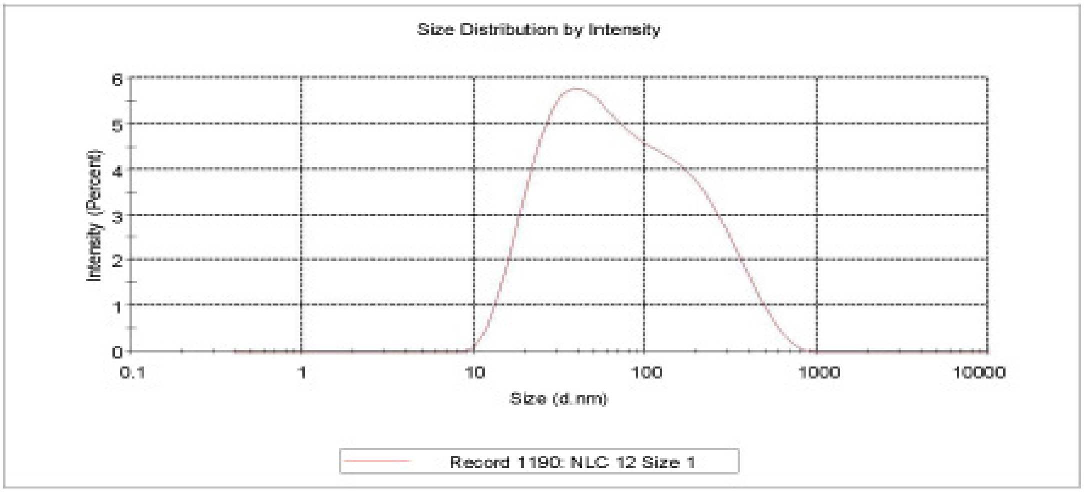
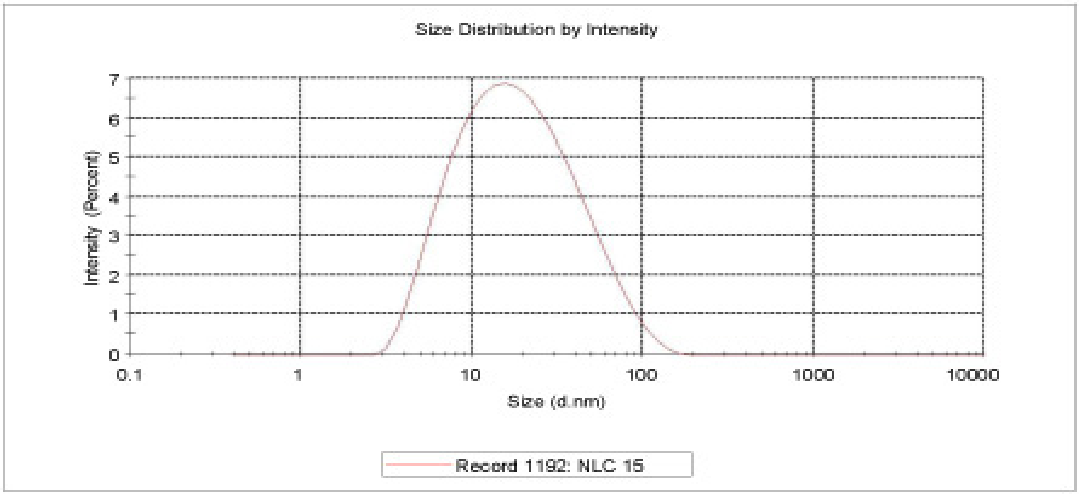


FIGURE 6A: PARTICLE SIZE DISTRIBUTION CURVE OF FORMULATIONS F3, F6 AND F9





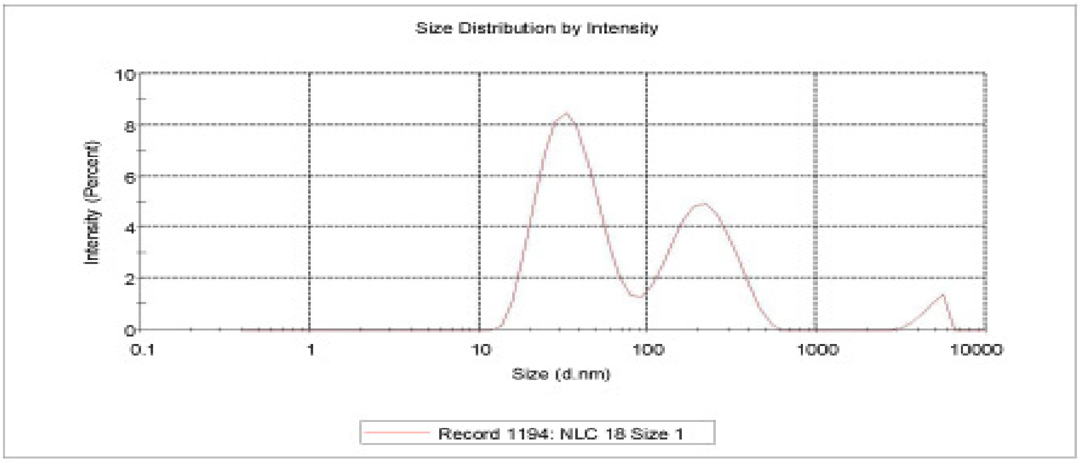
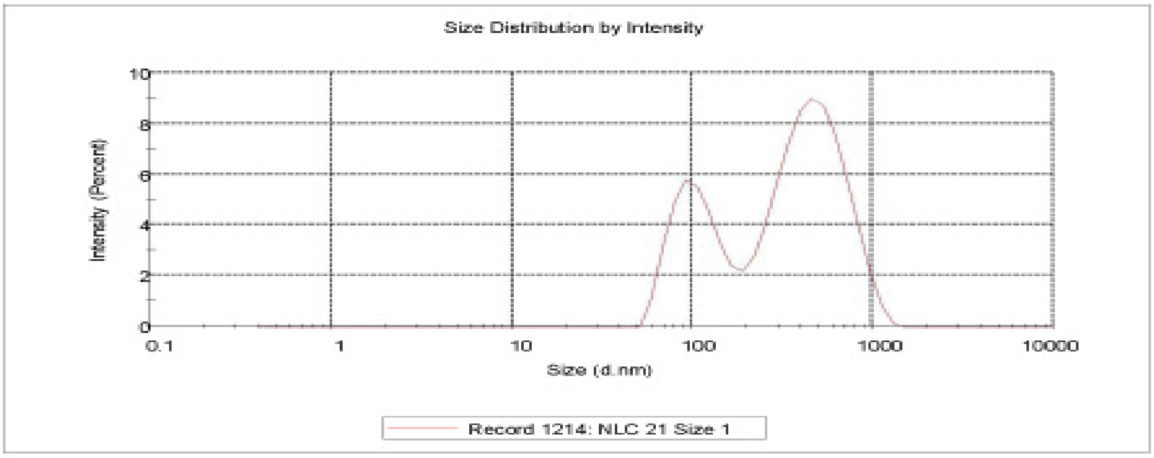
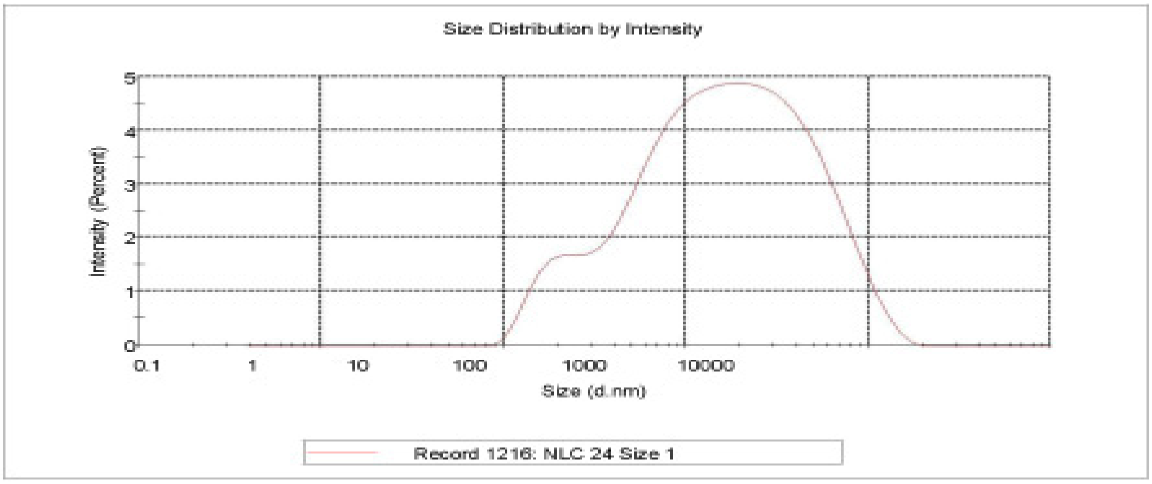


FIGURE 6B: PARTICLE SIZE DISTRIBUTION CURVE OF FORMULATIONS F12, F15 AND F18





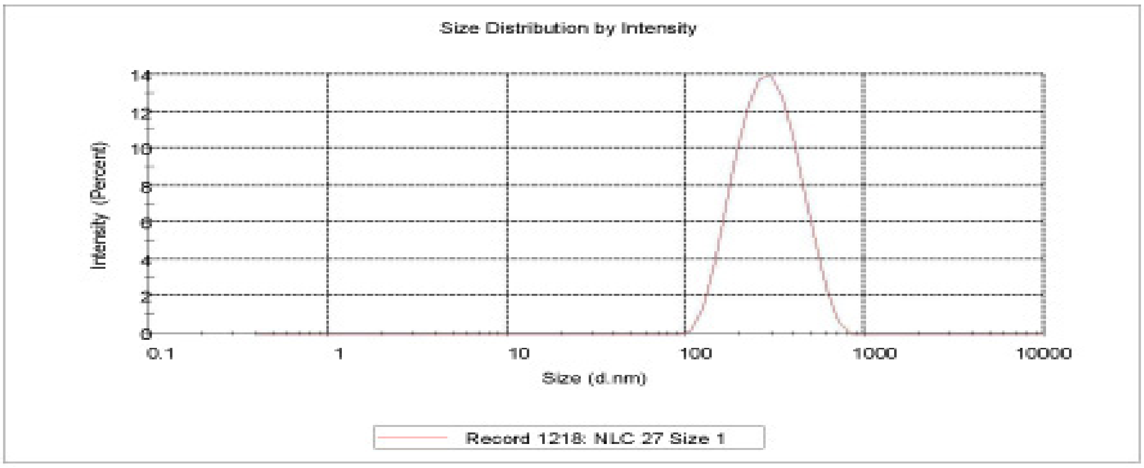
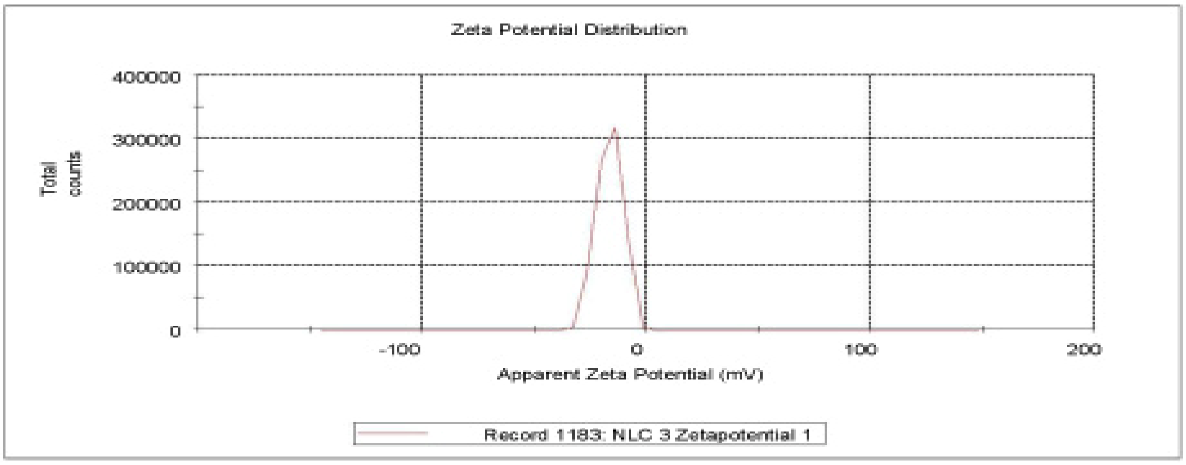
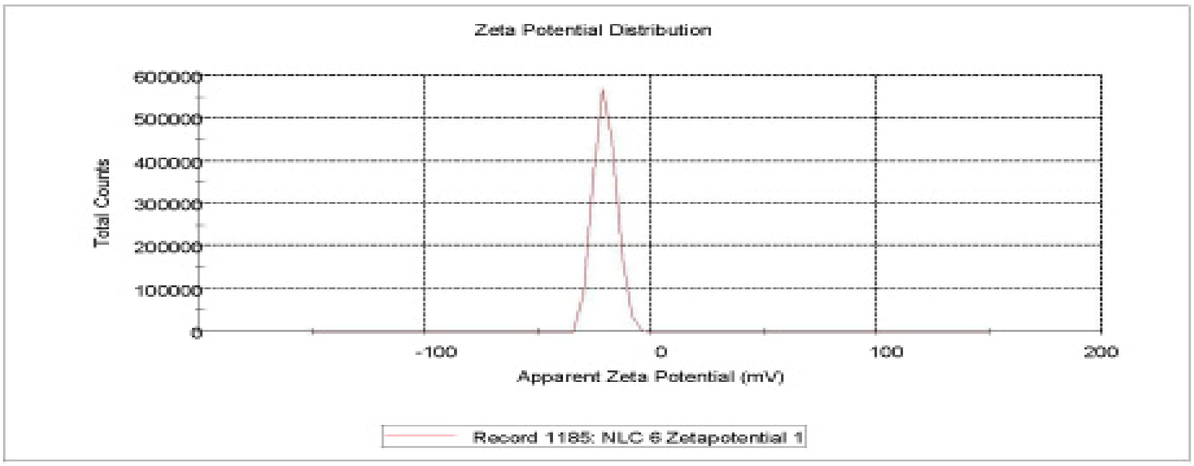


FIGURE 6C: PARTICLE SIZE DISTRIBUTION CURVE OF FORMULATIONS F21, F24 AND F27





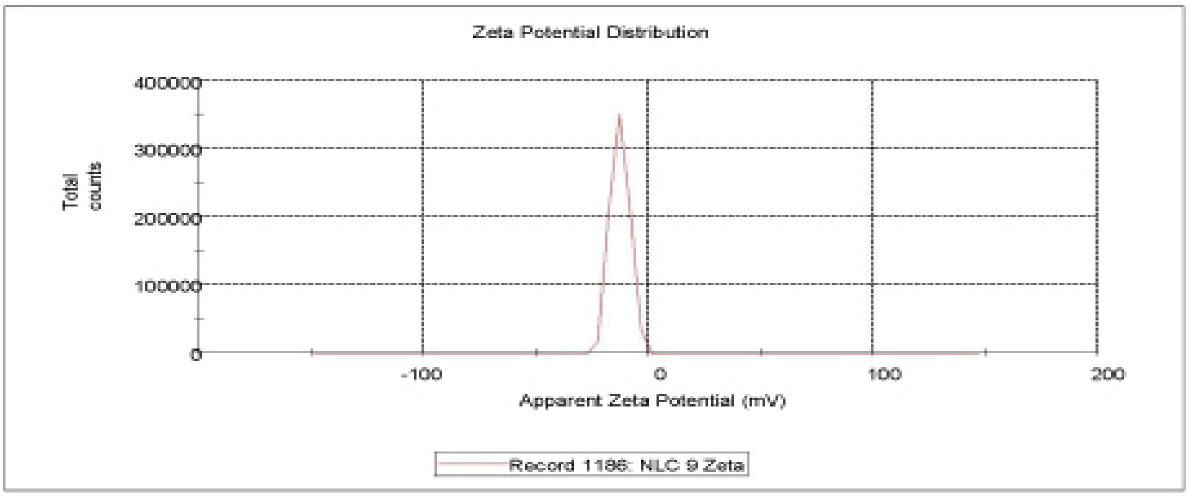


FIGURE 7 A ZETA POTENTIAL CURVE OF FORMULATION F3, F6 AND F9

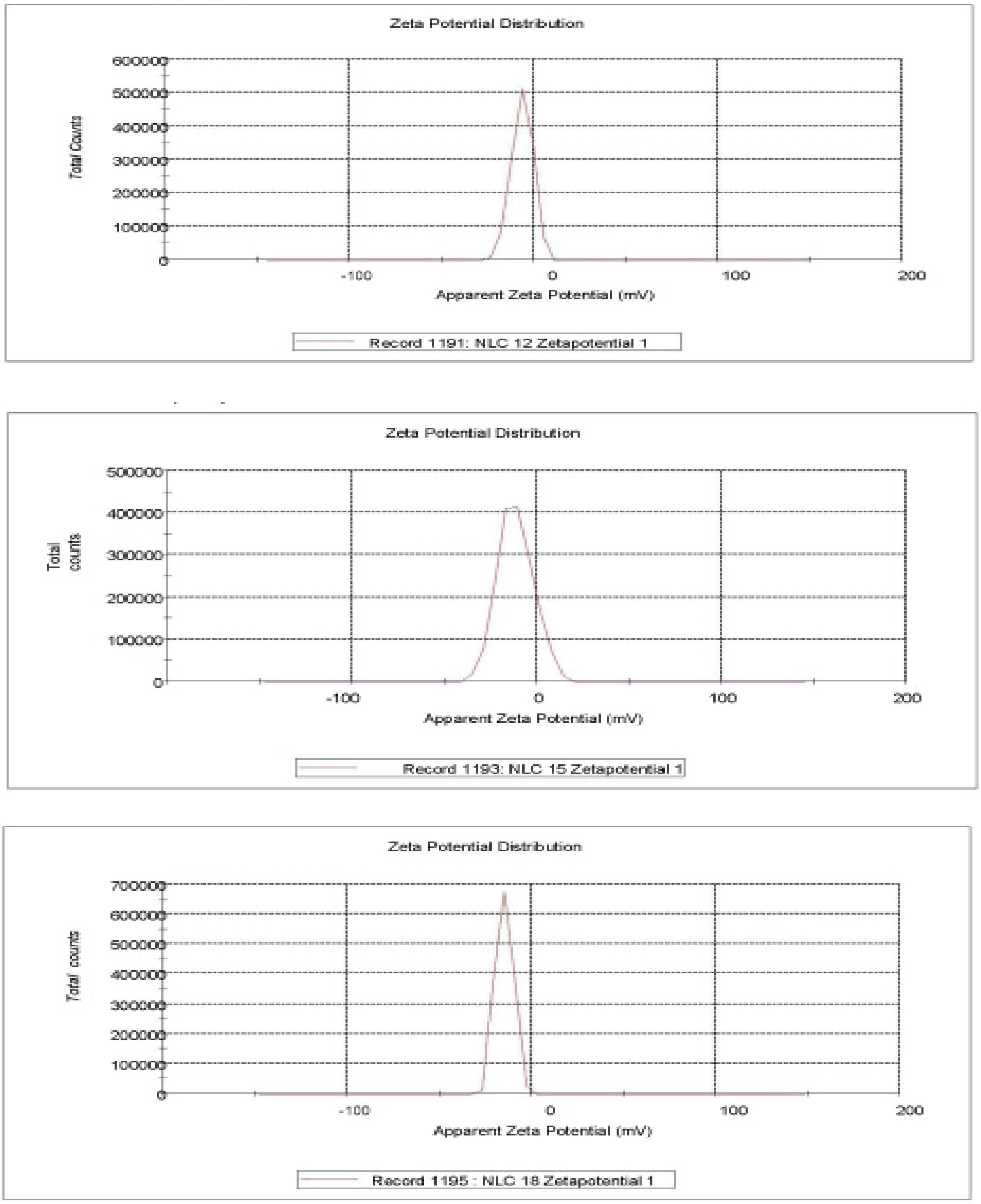
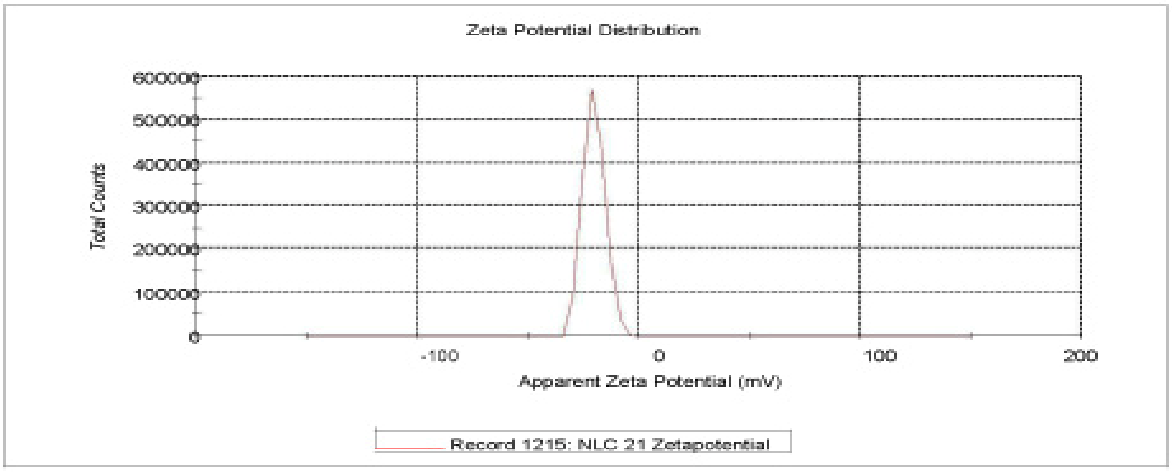
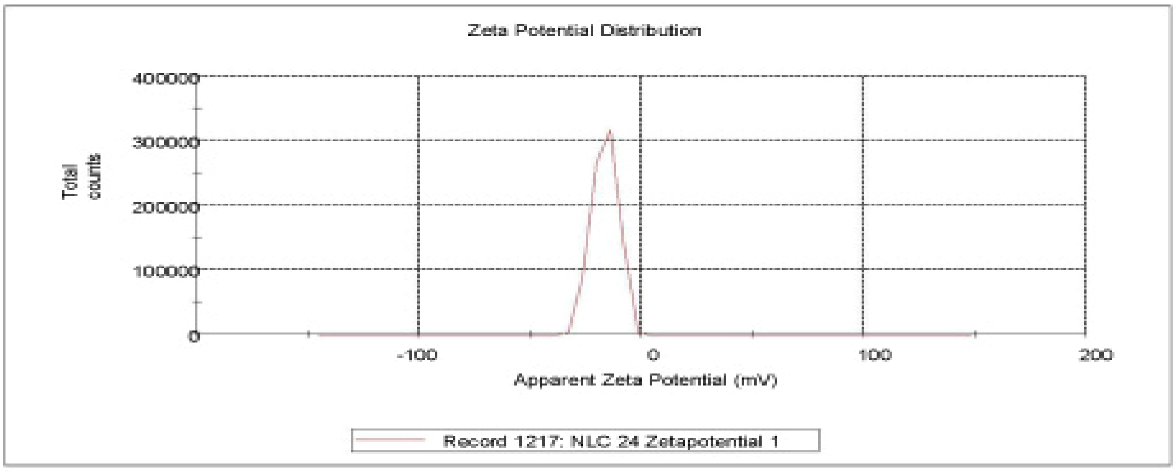


FIGURE 7B: ZETA POTENTIAL CURVE OF FORMULATION F12, F15 AND F18





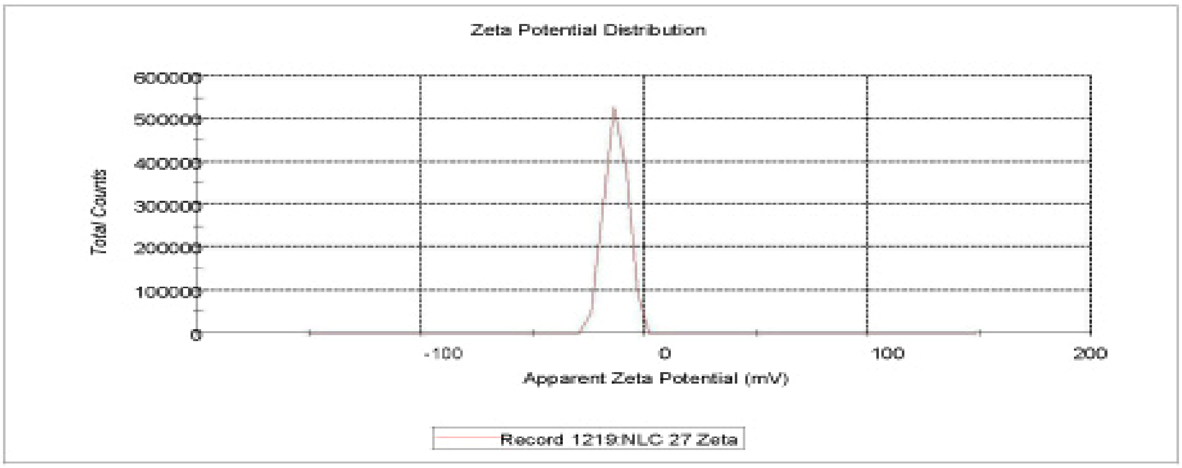


FIGURE 7C: ZETA POTENTIAL CURVE OF FORMULATION F21, F24 & F27

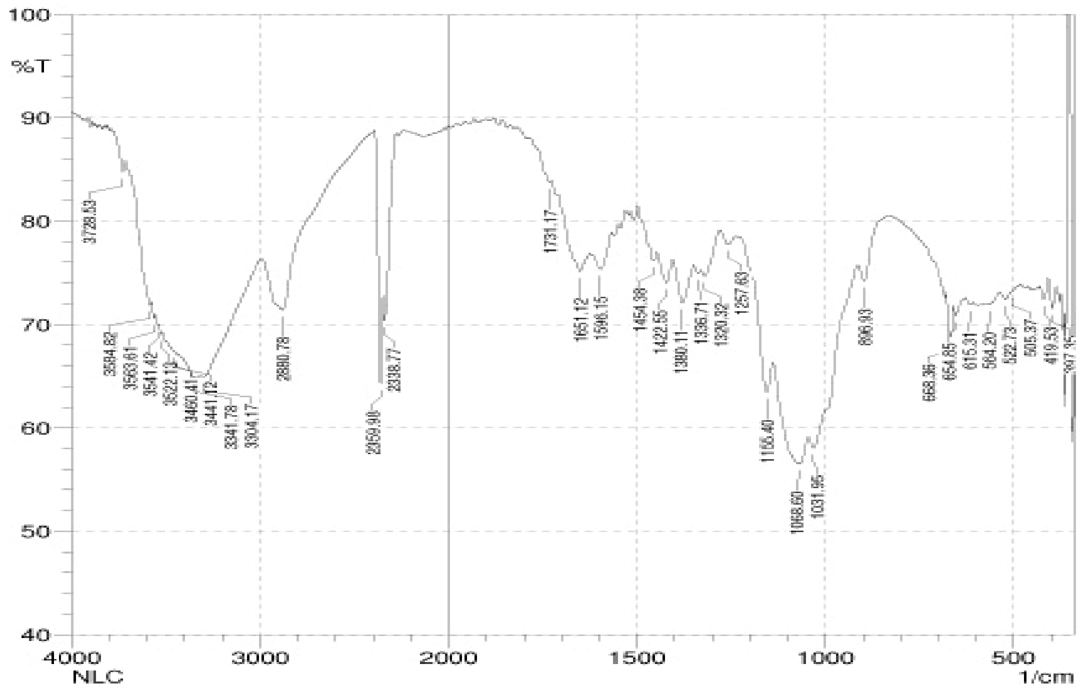


FIGURE 8: FT-IR SPECTRA OF BEST FORMULATION (F15)

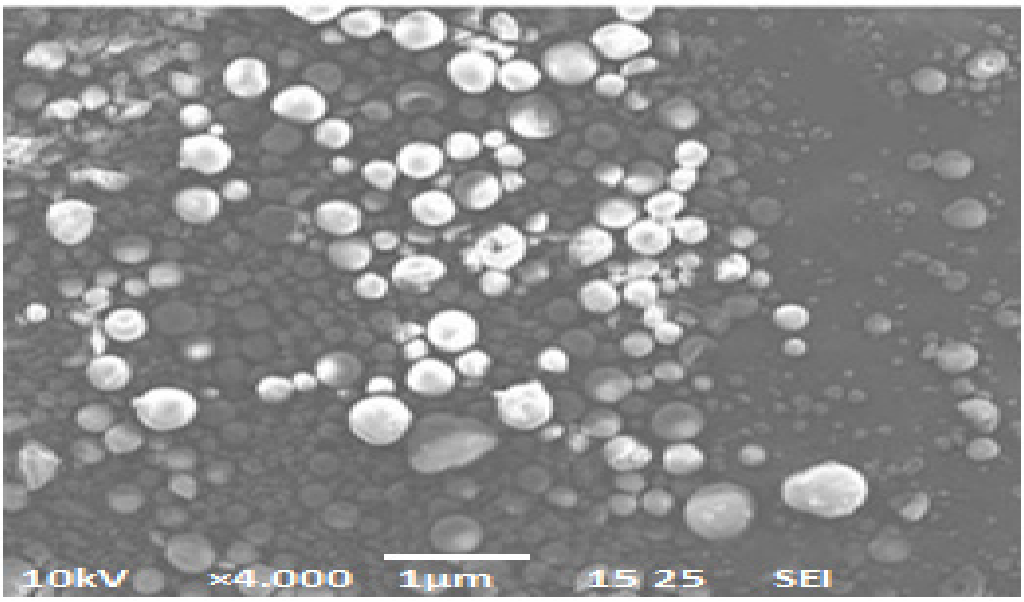


FIGURE 9: SCANNING ELECTRON MICROSCOPY OF BEST FORMULATION F15

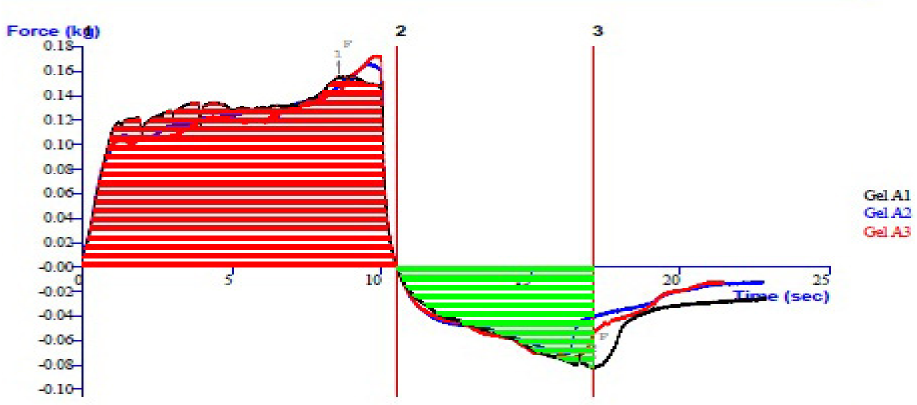


FIGURE 10A: TEXTURE ANALYSIS OF PLAIN HYDROGEL

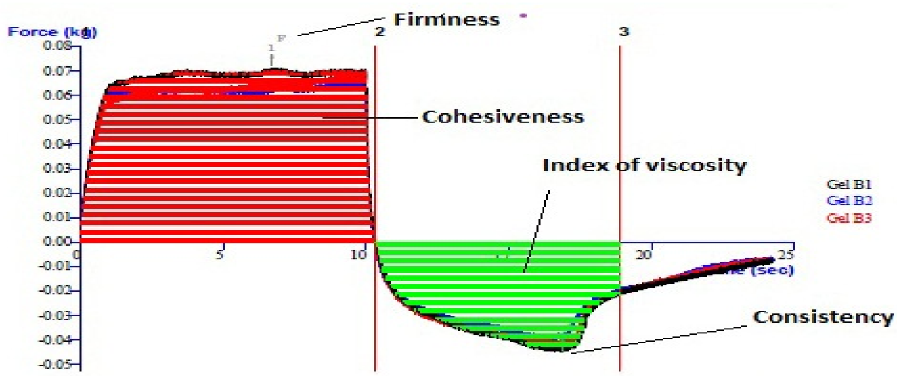


FIGURE 10B: TEXTURE ANALYSIS OF NLC BASED HYDROGEL

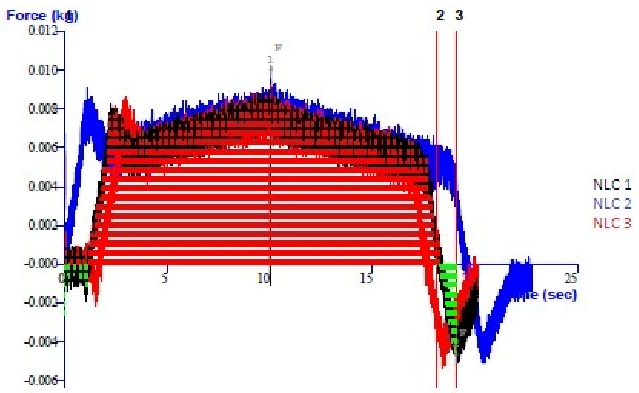


FIGURE 10C: TEXTURE ANALYSIS OF BEST NLC FORMULATION (F15)

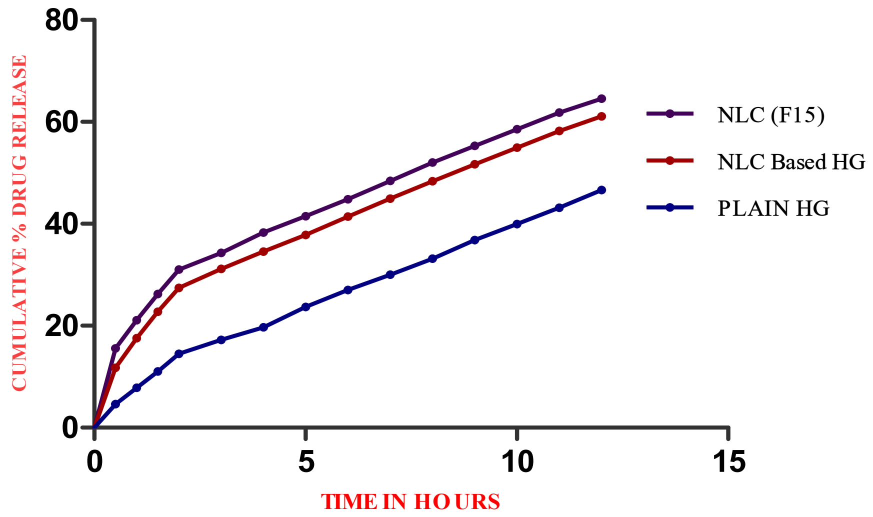


FIGURE 11: COMPARISION OF *EX-VIVO* PERMEATION OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL THROUGH EXCISED RAT SKIN

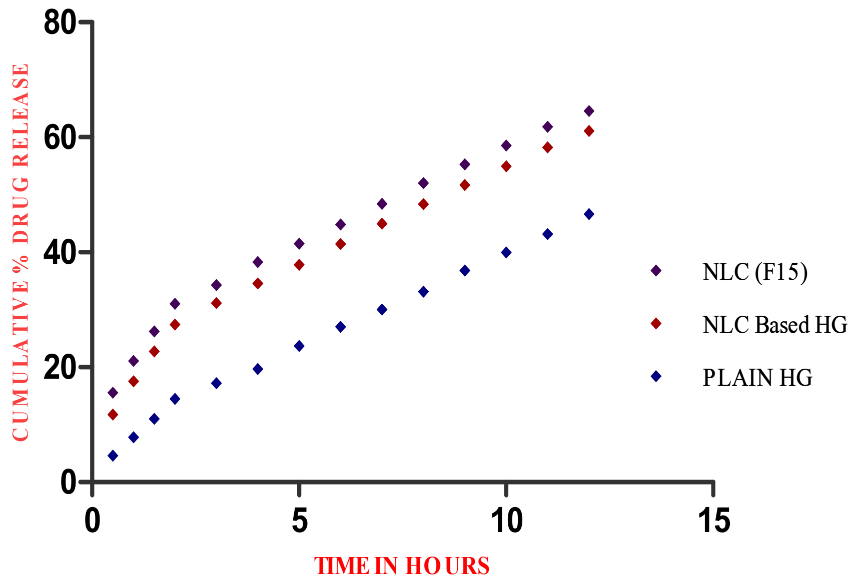


FIGURE 12A: COMPARISON OF *EXVIVO* ZERO ORDER RELEASE KINETICS OF OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL

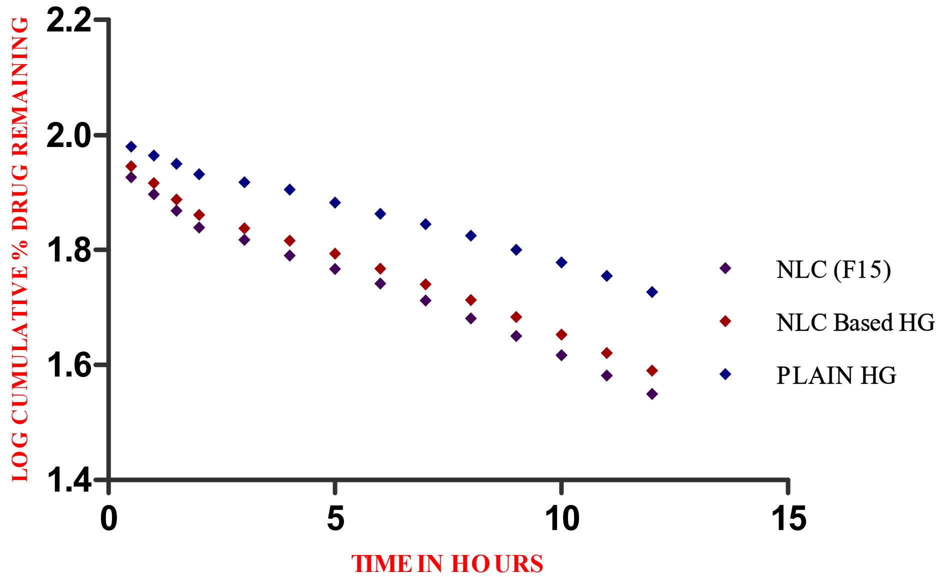


FIGURE 12B: COMPARISON OF *EXVIVO* FIRST ORDER RELEASE KINETICS OF OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL

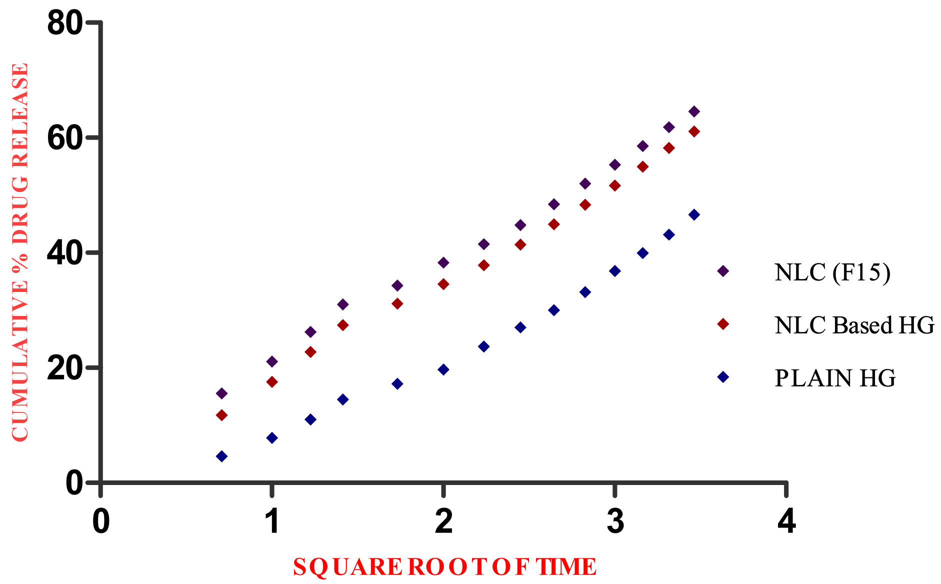


FIGURE 12A: COMPARISON OF *EXVIVO* HIGUCHI MODEL RELEASE KINETICS OF OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL

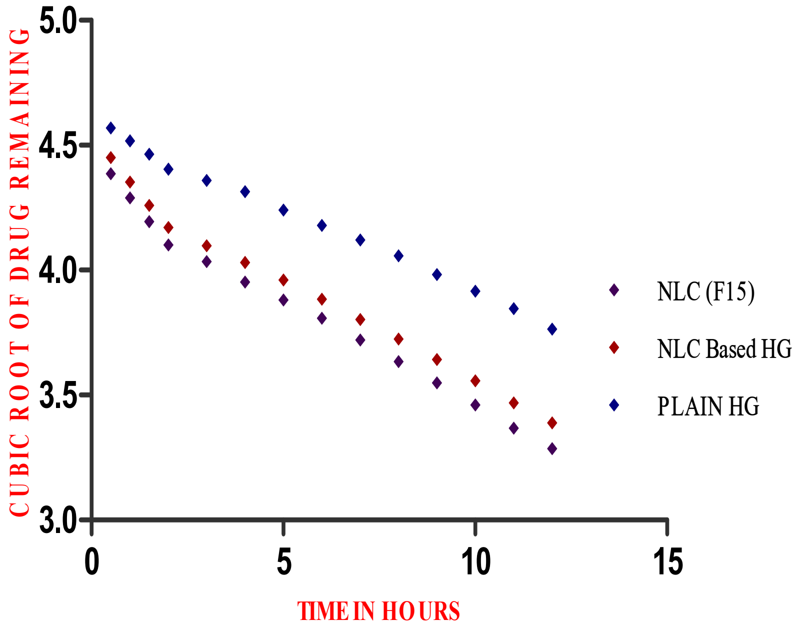


FIGURE 12A: COMPARISON OF *EXVIVO* HIXON CROWEL RELEASE KINETICS OF OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL

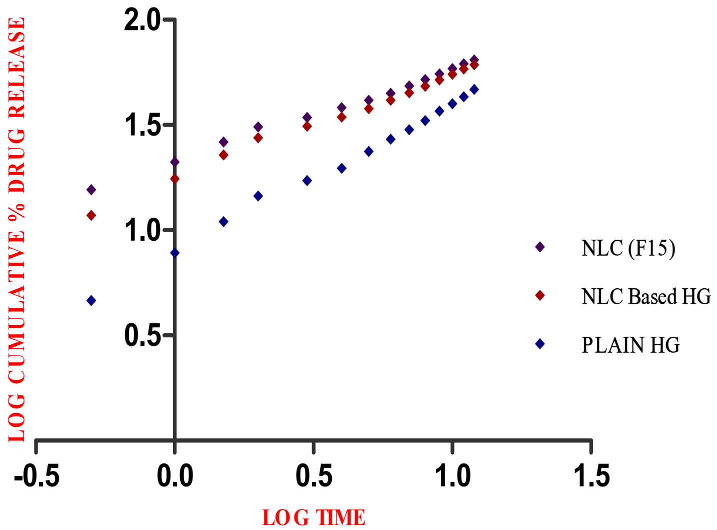
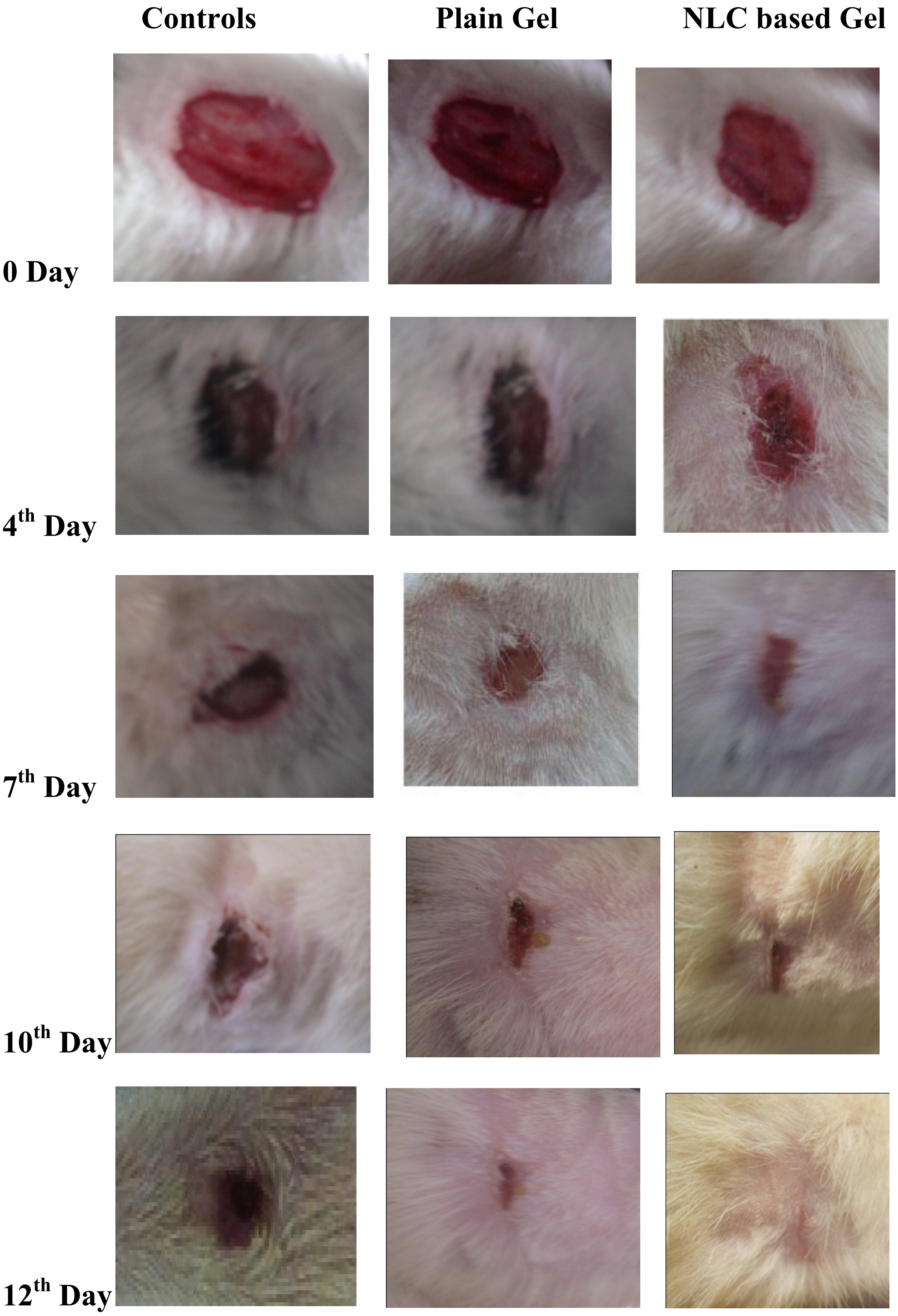
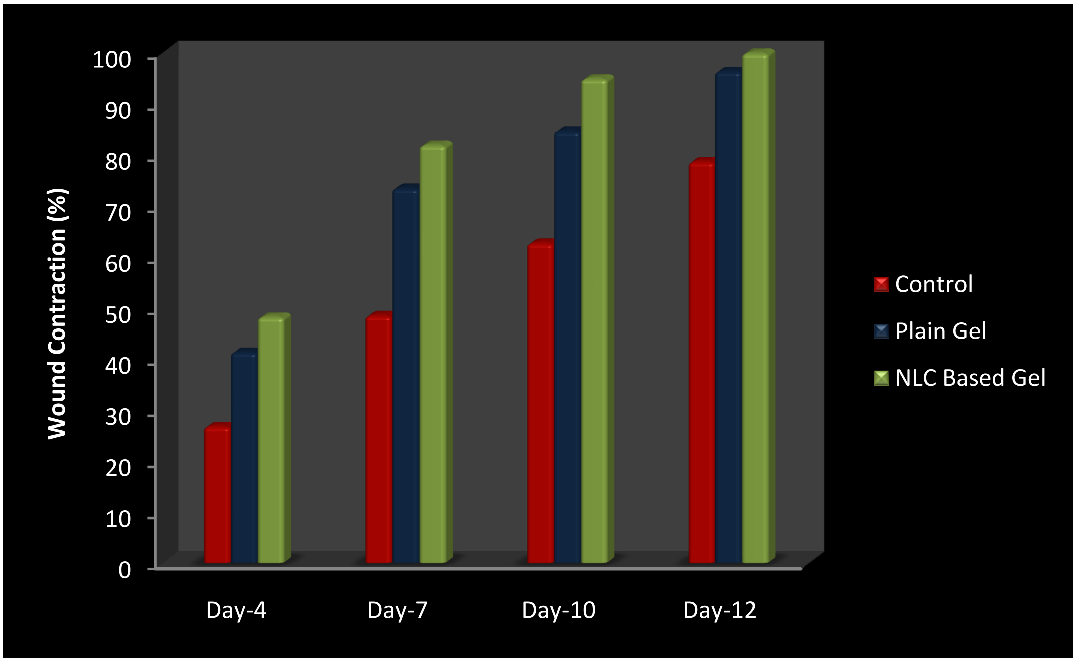


FIGURE 12A: COMPARISON OF *EXVIVO* KORS MEYER PEPPAS MODEL RELEASE KINETICS OF OF NLC, NLC BASED HYDROGEL & PLAIN HYDROGEL

FIFURE 13A:COMPARISION OF PERCENT OF WOUND CONTRACTION FOR IN VIVO WOUND HEALING EXPERIMENTS



FIFURE 13B:COMPARISION OF PERCENT OF WOUND CONTRACTION FOR IN VIVO WOUND HEALING EXPERIMENTS



CHAPTER –XI

SUMMARY AND CONCLUSION

CHAPTER - XI

SUMMARY AND CONCLUSION

- The purpose of this research was to develop a simvastatin loadednanostuctured lipid carrier based hydrogels as a potential dermatological formulation for diabetic wound healing with enhanced permeation, sustained drug release and enhanced drug release at the site of action.
- Infrared spectroscopic studies confirmed that there was no interaction between drug, lipids and excipients.
- Hot homogenization followed by ultrasonication were employed to produce NLCs using a range of solid lipids (stearic acid, Glyceryl mono stearate and compritol) , liquid lipids (capryol, oleic acid and GMO) and surfactants (Tween 80, phospolipon 90G).
- The entrapment efficiency increased with increasing the concentrationof lipids and phospholipon 90G and the formulations containing Phospholipon 90G at 3% showed higher entrapment irrespective of the lipids composition.
- The mean particle size of simvastatin loadednanostructured lipid carrierformulations F1-F27 showed a suitable particle size in the range of 14.06 nm-235.2 nm and thepolydispersity index were less than 0.5, whichindicated a relative homogenous dispersion.
- The zeta potential of simvastatin loaded NLC showed a negative surface charge(-11.63 mV to -23.4 mV)which revealed that the prepared NLC have sufficient charge and mobility to inhibit aggregation of particles.
- Theinvit*ro*release studies displayed a similar biphasic drug release pattern with a burst release within 2 hours followed by sustained release at 12 hours.

- The best formulation F15 containing stearic acid and oleic acid showed the spherical shaped particles in the size of 14.06 nm , PDI (0.347) and zeta potential (-13.6 mV) with acceptable EE of 40.64% and invitro release of 70.85% after 12 hrs and which is successfully incorporated into chitosan HG.
- Texture Anayser TA- XT Plus confirmed the desired properties of firmness, adhesiveness and viscosity of prepared NLC based HG.
- The ex vivo release studies and drug deposition studies indicated that the prolonged drug release and better penetration capacity of NLC based HG as compared to NLC dispersion and plain HG. Chitosan was also playing an important role for the prolong release of simvastatin up to 12 hrs.
- Invitro release kinetics revealed that the classical Fickian diffusion was the release mechanism for NLC dispersion and anomalous non-Fickian for both NLC based HG and plain HG.
- The results of the invivo diabetic wound healing study indicate that simvastatin loaded NLC based HG promote the healing in diabetic wounds with faster and higher wound contraction rate as compared to NLC dispersion and plain HG. Skin irritation study was also shown that no irritation on skin.

Hence, it wasconcludedthat the hot homogenization was a useful method for the successfulincorporation of simvastatin inNanostructured lipid carrier with higher EE, sustained drug release and smaller particle size.The result indicates that NLC Based HG enhanced the permeation, sustained the drug release and enhanced the drug release at the site of action due to submicron size of lipid nanoparticles and gel matrix of chitosan HG. Thus the NLC based HG can be used as novel drug delivery carrier for skin targeting of simvastatin for its diabetic wound healing action.

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ANNEXURE

ANNEXURE

Investigator declaration

- I certify that I have determined that the research proposal herein is not unnecessarily duplicate of previously reported research.
- I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedures.
- For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
- I will obtain approval from the IAEC / CPCSEA before initiating any significant changes in this study.
- Certified that performance of experiment will be initiated only up on review and approval of scientific intent by appropriate expert body (institutional scientific advisory committee / funding agency / other body (to be named)
- Institutional biosafety committee (IBC) certification of review and concurrence will be taken (required for studies utilizing DNA agents of human pathogens)
- I shall maintain all the records as per format (Form D)

Signature

(S.Sudhakar)

N. Chidambaranathan

Name of Investigator

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.